Variation in captive and wild populations of the barn owl *Tyto alba alba* (Scopoli)

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ABSTRACT

Barn owls *Tyto alba alba* have undergone a population decline in Britain since the 1930s; the release of captive-bred barn owls has been a popular response to the perceived problem. A loss of genetic variability is predicted from available demographic data for both captive and wild barn owl populations; this study was initiated to assess the suitability of protein electrophoresis and quantitative variation for monitoring genetic variation in barn owl populations.

A total of 304 samples were obtained over three years, from barn owls from two wild and three captive populations in Britain. Nest boxes were installed during the winter to facilitate the sampling of wild barn owls during the breeding season; they were caught by the use of hand held nets or a box trap designed for use with the nest boxes. Blood samples and measurements were taken from each bird. Non breeding barn owls were assigned a sex on the basis of plumage characteristics; a discriminant function analysis gave an accuracy of 86.5% when tested on adults of known sex. Karyotyping and steroid hormone assays proved unsatisfactory for sex determination in this study.

Protocols for horizontal starch gel electrophoresis were developed by modification of existing systems; 11 proteins (MPI, GPI, PGM, SOD, 6-PGD, MDH, LDH, EST-S, Hb, Tf, Pt) were monomorphic in 229 blood samples. The only phenotypic variation in blood proteins was an additional band occurring in 21 breeding females, interpreted as albumen present transiently in the blood during egg production.

Quantitative traits were assessed for their suitability for heritability analysis (measurement error, intercorrelations of traits, sexual dimorphism and population differentiation); fluctuating asymmetry (FA), and two possible predictors of body condition based on tarsus and weight data, and a blood parameter (packed cell volume) are also discussed.

Heritability in one wild population were calculated by parent offspring regressions and analysis of variance of sibling data. Heritability estimates were characterised by large standard errors; the problems of estimating heritability in wild populations are discussed. Heritability of tarsus length by mean offspring / mid-parent regression (±S.E.) was 0.64 ± 0.27; the genetic coefficient of variation was 2.40%. It is argued that the genetic coefficient of variation, rather than heritability, is the appropriate statistic for inter-population comparisons of heritable quantitative variation; the barn owl population had a level of genetic variation for tarsus length comparable to that demonstrated in other avian studies. The merits of isozyme and quantitative genetic approaches to monitoring genetic variation are discussed.

DECLARATION

I hereby declare that this thesis was composed entirely by myself, and the work is my own except where otherwise acknowledged.

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Chapter 1

A GENETIC APPROACH TO BARN OWL CONSERVATION

1.1: Introduction

This project was initiated in response to concerns over the possible genetic consequences of a declining wild barn owl population, and of the release of captive bred barn owls in Britain (Hanna, 1992). The possible genetic consequences of a declining wild population include inbreeding and a loss of genetic variation, resulting in a reduction in the population's potential to adapt to a changing environment. Recognising the value of a method for directly monitoring levels of genetic variation in a population (Lande and Barrowclough, 1987, and see section 1.3 this thesis), two commonly used techniques for assessing genetic variation (protein electrophoresis and quantitative analysis) were employed, with the aim of comparing their merits and weaknesses for monitoring genetic variation in barn owl populations.

In this chapter, the background to the barn owls predicament in Britain is described in section 1.2; genetic issues are introduced in 1.3 and the range of available techniques for genetic monitoring are outlined in 1.4. The objectives of this project are set out in 1.5. In chapter 2, demographic factors directly related to gene flow in barn owls are reviewed as supporting evidence of the possible genetic consequences of a population decline. The barn owl in Britain is then placed in its global context through a discussion of the distribution of *T. alba alba* and other sub-species of barn owl, and the relevance of this for conservation management of barn owls in Britain is discussed.

The selection and sampling of populations in this study to compare the chosen methods for monitoring genetic variation is described in chapter 3, and how these birds were assigned a sex is detailed in chapter 4. Variation in quantitative traits is introduced in chapter 5, leading to a heritability study in a wild population in chapter 6. A study of isozymes by starch gel electrophoresis is described in chapter 7; the two approaches to monitoring genetic variation in barn owl populations are compared in chapter 8.

1.2: Barn owls in Britain: the perceived problem

In Britain, the barn owl *Tyto alba alba* was once a common countryside resident (Shawyer, 1987); its striking appearance and habit of often nesting in close proximity to man attracted particular attention to this species in rural areas. Preying mainly on small mammals, it earned the reputation of being 'the farmers friend', as although its most frequently taken prey is the common vole *Microtus agrestis* (Bunn et al., 1982), it also exploited the mice and rats typically found around farm buildings before modern intensive farming practices were introduced. Today the frequent appearance of its image in a variety of contexts, such as on greetings cards or in publicity for conservation projects, testifies to its popularity; it continues to be a widely recognised (though rarely seen) bird.

Although there are no accurate figures for historical changes in barn owl numbers, there is a general consensus of opinion amongst local natural history societies, farmers and ornithologists that the barn owl has been experiencing a decline in numbers since the 1930s which accelerated in the 1960s (Taylor, 1994 16.1), correlating with some major changes in farming practice resulting in habitat loss and pesticide poisoning. A review of population estimates for the period 1982-1985 placed the current British population at around 4400 breeding pairs (Gibbons et al., 1993).

In response to the observed decline, many voluntary groups and individuals in Britain began to breed barn owls in captivity with the intention of releasing the progeny into the wild. Warburton (1992) claims that the origins of the 'captive breeding and release movement' lie with his rehabilitation of two orphaned barn owls in 1965; he began the 'British Owl Breeding and Release Scheme' (BOBARS) in 1972, and other groups became active around this time. Barn owls breed readily in captivity if provided with abundant food, and the captive population may now far exceed the native wild population in Britain; David Ramsden of the Barn Owl Trust (BOT), Devon, estimated a captive population of 20 000 to 30 000 barn owls in 1992 (reported in Cayford and Percival, 1992). The releases were uncoordinated and at the time there were no legal constraints to the practice other than those which govern aspects of animal welfare, hence there are no comprehensively compiled records on the extent of early releases throughout the country. It is estimated, however, that around 3000 barn owls were being released annually by 1989 (Nature Conservancy Council Ornithology note no. 14, 1989), with the practice gaining publicity through newspaper reports and television coverage. Cayford and Percival (1992) reported an increase in the number of closed rings sold for barn owls by the British Bird Council (BBC) from 1000 in 1983 to 6000 in 1990. They consider it likely that most barn owls released during this period were not fitted with BBC rings, and so the actual number of released barn owls could be far higher.

Many (but not all) of the released birds were being fitted with British Trust for Ornithology (BTO) rings at this time; of a large sample (>500) of released captive bred barn owls ringed during the period 1982-1987, 10% of first year and 15% of adult birds survived, compared to 19% and 55% for wild birds ringed in the same period. (Cayford and Percival, 1992). The high mortality of captive bred birds shortly after release was placing a large burden on the BTO record scheme, and opened questions concerning the cruelty of releasing birds unfit for an independent life, of the release of birds in unsuitable areas, or by methods which gave them little opportunity to adjust to the wild from a captive environment.

Although the majority of released birds appeared never to reach breeding age, and therefore failed to become part of the wild breeding population, there remained the possibility that released birds could be having some impact on the remaining wild population. Increased competition or the inadvertent introduction of disease could be a burden on an already stressed wild population, and introduced birds could be a confounding effect in monitoring wild populations, as by masking the decline of wild breeding birds, habitat deterioration could occur without evidence that this was detrimental to the wild population. Also, as some release schemes did achieve at least local success in establishing breeding birds, the possible genetic impact of the releases was questioned (Hanna, 1992). These concerns brought the entire practice of releasing captive bred barn owls into question, and Cayford and Percival (1992) called for a change in legislation that would allow control over the extent of barn owl releases.

Recognition that the practice of releasing captive bred barn owls in Britain could be both detrimental to the existing wild population, and potentially cruel to the released individuals, resulted in the addition of the barn owl to Schedule 9 of the Wildlife and Countryside Act 1981; the order came into effect on 25th November 1992. From this date, it became an offence to release barn owls in Britain, except under licence, or to allow them to escape (S. M. Davies, in Hanna, 1992). The legislative framework was therefore established that could contribute to a reasoned and co-ordinated conservation strategy for this species.

The priority in conservation problems of this kind should be to identify and alleviate the causes of the wild population decline; management options towards the goal of establishing self-sustaining populations include habitat improvements, providing artificial nest sites, increasing productivity and survival through supplementary feeding, or augmenting the population with captive bred or wild translocated individuals. A successful population management strategy for barn owl conservation would encompass demographic, stochastic and genetic issues, and although non-genetic factors may be considered the most immediately

relevant to survival in a declining wild population (Lande, 1988), it is widely recognised that genetic issues should be taken into consideration in long term population management plans (Lande and Barrowclough, 1987), and are particularly important in the management of captive breeding programmes (Temple and Cade, 1988). Genetic issues are therefore discussed in the following section.

1.3: Conserving genetic variability

The broad aim in genetic management is to maintain genetic diversity throughout the genome (Avise, 1994). In small (often captive) populations, management to maintain overall genetic diversity reduces the risk of inbreeding depression- the well documented reduction in fitness (as described by fecundity, survival and growth rate) which occurs when a naturally outbreeding population increases its level of inbreeding, resulting in the expression of deleterious recessive alleles (Templeton and Read, 1983). Inbreeding depression has been demonstrated in wild populations of great tits *Parus major*, where nestling mortality was found to be significantly higher in inbreeding than in outbreeding pairs (Greenwood, 1987) and in song sparrows *Melospiza melodia* (Arcese, 1989; Keller et al., 1994) where inbred birds were under-represented in the survivors of a population crash which occurred over a severe winter. In larger (wild) populations the emphasis is placed on the longer term aim of maintaining variability to allow adaptation in a changing environment (Avise, 1994). Evidence from wild populations that a reduction in genetic variability results in a decrease in the ability to adapt to change is more difficult to find; a study of the cheetah major histocompatability complex (MHC) suggests a causal relationship between low variability and the viability of the population (O'Brien and Evermann, 1988), and several other studies find a correlation between low heterozygosity and fitness, although this is not always the case (see Avise, 1994 for a review). Loss of genetic diversity is related to population size, such that in the absence of factors acting to maintain variation, the expected loss of heterozygosity at single loci, or of additive genetic variance in quantitative traits is $1 / (2 N_e)$ per generation (Lande, 1988). One consequence of the relationship between N_e and the loss of genetic variation is the possibility that the release of captive bred individuals may be counterproductive even if it results in an overall increase in numbers. If released individuals are derived from a small breeding group, for instance, the inbreeding coefficient for the wild population may actually be increased by their incorporation into the wild population (Ryman and Laikre, 1991).

It should be noted that the emphasis in genetic management is placed on maintaining the levels of genetic variability that are naturally present in a viable population of the particular species of interest; the aim is not simply to maximise variability by the introduction of genetically divergent stock. Such an approach could itself be counterproductive, resulting in the loss of

local adaptation and unique local characteristics, or the possible loss of intrinsic adaptation by the disruption of coadapted gene complexes, described by Templeton (1986) as 'outbreeding depression'. How these factors influence management decisions concerning the choice of individuals for captive breeding or translocation is discussed more fully in chapter 2.

One approach to managing a population to maintain diversity is to monitor the population size, estimate N_e, and to predict the effect on genetic variability as N_e changes. Although this approach is adequate for short-term predictions concerning the loss of diversity as populations change, inaccuracies in the estimation of N_e and of the processes which affect genetic variation may be compounded over several generations, reducing the confidence in long term predictions (Lande and Barrowclough, 1987). This is particularly relevant to wild barn owl populations, for which it is notoriously difficult to obtain accurate census data. Individuals in a population are distributed at low density; they are secretive and largely nocturnal. In addition, populations naturally fluctuate in accordance with three to four year fluctuations in prey availability, making estimates derived from a single year difficult to interpret without additional data on prey dynamics.

The challenge is therefore presented to directly assess levels of genetic variability in a population in addition to obtaining census data for long term management plans (Lande and Barrowclough, 1987). The first problem encountered here is that the amount of quantifiable genetic variation present does not relate simply to fitness at the population level. The level of variability that corresponds to homeostasis (developmental stability) varies greatly among species, and this is reflected in different average levels of polymorphism and heterozygosity in viable natural populations (Merola, 1994). Populations which experience prolonged periods of low levels of inbreeding reduce the population genetic load through selection against deleterious genotypes, and are then less likely to suffer inbreeding depression under intense inbreeding. Low levels of isozyme variation, for instance, have been correlated with a polygynous mating system, large and highly mobile species, dietary specialists and fragmented populations (Negro and Hiraldo, 1994); Merola (1994) suggests that low variation is normal in large terrestrial carnivores. Low levels of variation may not be unusual in species which naturally have a low N_e, and this is therefore not necessarily a cause for concern in population management.

The implication of this is that little can be learned from a single assessment of genetic variability; a control population of some kind is required. This could be a conspecific, non endangered population (Sherwin et al., 1991); levels of variation in captive barn owl populations could be compared with the situation in a self-sustaining wild population, for

instance. The alternative approach is to monitor a population for change over a number of generations.

The second problem encountered in monitoring genetic variation at the population level is that available genetic methods sample only a small proportion of the genome, and there is frequently a lack of concordance in the levels of variability revealed by the different methods (Schaeffer et al. 1987; Ferguson et al., 1991; Hillis et al., 1991; Crawford et al., 1992; Helgason, 1993; Cheverud et al., 1994). Lande and Barrowclough (1987) describe how the evolutionary dynamics of single locus neutral polymorphisms and quantitative traits are quite different; if variation in the former is lost due to inbreeding in a severe bottleneck it would take many generations to recover (10^5 to 10^7), whereas quantitative variation could recover an order of magnitude more rapidly. Levels of variation may therefore reflect demographic events in the far distant past, and variation as assessed by one method may not be a good predictor of variation assessed by an alternative method (Hartl and Pucek, 1994).

It follows that the suitability of a particular method should be carefully assessed before a large scale project is embarked upon (Avise, 1994); in this case a technique is required which is informative at the inter-population level, both for interpopulation comparisons, and for monitoring one population over several generations. Methods of assaying genetic variation are described in the following section.

1.4: Methods for assaying genetic variation

Early approaches to assaying genetic variation in birds were limited to phenotypic differences in easily accessible characters such as plumage coloration or body dimensions.

In some cases, the traits proved to be under the control of a single locus, and patterns of Mendelian inheritance could be traced. With the plumage morphs of lesser snow goose *Anser caerulescens*, for instance, the blue colour is incompletely dominant to white (Cooke and Cooch, 1968). The direct relationship between phenotype and genotype makes this an attractive approach to studying genetic variation, but is extremely limited in its applicability as few species exhibit such polymorphisms.

Measurements of quantitative traits such as wing or tarsus lengths, in contrast, are under the control of several loci, and the phenotype is the result of the additive effects of genes and an environmental component which blurs the distinction between genotypes. As simple patterns of inheritance are not apparent, relatively large samples of individuals of known pedigree are

required, so that the additive genetic variance can be determined through the resemblance between relatives. This approach to assaying genetic variation is therefore subject to problems of interpretation. Quantitative variation is usually described in terms of narrow sense heritability (the ratio of additive genetic variance to phenotypic variance). The appeal of studying quantitative traits lies with the ease with which data are obtained from any species, and with the fact that the majority of traits that are of evolutionary interest are of a quantitative nature (see chapters 5 and 6).

Karyotyping provides a more direct approach to assaying genetic variation; chromosome variation may be numerical or structural. Developments in chromosome staining techniques to produce distinctive banding patterns allow homologous chromosomes to be paired, and mutations such as deletions, inversions or insertions to be detected. Closely related species have similar karyotypes, but more distantly related taxa differ in their chromosome number. Typical diploid numbers (2n) for falcons and hawks, for example, are 50-52 for Falconidae and 60-80 for Accipitridae (Shields, 1987). Intra-specific variation has also been recorded for some avian species; the white throated sparrow *Zonotrichia albicollis*, for instance, is polymorphic in two macrochromosomes and the differences correlate with plumage variation. Most avian species studied so far have a large number of microchromosomes, which makes karyotyping of birds a difficult task; they are easily lost in chromosome preparations, and so the diploid number for many species remains uncertain. Shields (1987) noted that only around 6% of modern bird species had been karyotyped, and few were the subject of detailed banding studies.

The development of molecular genetic techniques over the last 30 years has opened up the field of population genetics; it is now possible to quantify genetic variation at all levels of organisation, from differences between closely related individuals to distantly related taxa. As each method samples different parts of the genome, which may evolve at quite different rates (Wilson et al., 1987), the most appropriate technique needs to be identified according to cost effectiveness and the level of differentiation appropriate for each study. Protein electrophoresis and microcomplement fixation assay gene products, whereas other techniques may directly assay variation in DNA.

Microcomplement fixation gives a measure of divergence between species based on the immune response which occurs when antiserum raised against a purified protein from a reference species is introduced to the diluted plasma of the test species. The amount of cross-reactivity between antibody and antigen gives a distance measure between the species. This

method is feasible for closely related species or distant taxa, but is most suitable for intermediate taxonomic levels (Burrin, 1986).

Protein electrophoresis separates proteins on the principle that proteins which differ in their net electrical charge migrate at different rates when a current is applied across a supporting medium such as a starch gel. The genetic basis of the protein variation can be confirmed if pedigree data are available, and so from a small tissue sample heterozygosity and polymorphism for a number of loci can be simply determined. Despite evidence that there may be selective differences among some isozymes (Zera, 1987; Powers et al., 1991), protein electrophoresis is usually assumed to assay effectively neutral single locus polymorphisms when the population size is small (10s to 100s of individuals) as the effects of drift would be expected to outweigh the impact of selection unless the pressure was severe (Kimura, 1983, Ohta 1992, see also chapter 7 this thesis). Protein polymorphisms are most suited to studies of con-specific populations or closely related species, although the presence or absence of particular genotypes may be informative at higher taxonomic levels (Avise, 1994).

Of the DNA techniques, DNA-DNA hybridisation is most suitable to studies of species which diverged around 2-100 million years ago (Avise, 1994); this technique involves hybridising strands of single copy DNA from two test species and measuring their degree of similarity by the thermal stability of the hybrid DNA; genetic distances of closely related species are close to zero with this method. Other techniques involve cutting DNA into different fragment lengths by the use of restriction endonucleases- enzymes which cleave DNA wherever particular oligonucleotide sequences occur. Many enzymes are available with different recognition sequences, creating different restriction fragment length polymorphisms (RFLPs). Applied to mitochondrial DNA, this gives data on variation in genetic material which is maternally inherited in animals; nuclear genetic data may be obtained from applying the same technique to single copy DNA. This approach is suitable at the level of con-specific populations. Hypervariable regions of DNA reveal banding patterns which distinguish individuals, hence 'fingerprinting' depends on variation in minisatellite DNA.

DNA sequencing, in which the actual sequence of nucleotides is inferred, is potentially useful at any taxonomic level by the appropriate choice of sequences to study, but is usually applied to higher taxonomic levels as it would be expensive and time consuming to investigate many individuals in a population study.

1.5 Objectives of this study

Of the range of available molecular and non-molecular genetic techniques available, Lande and Barrowclough (1987) highlight protein electrophoresis and the genetic analysis of quantitative traits as suitable candidates for population monitoring. These methods are chosen for this study as protein electrophoresis is an appropriate and cost-effective measure of inter-population variation which has been successfully employed in a number of avian population studies (e.g. in eagle owls *Bubo bubo* Radler, 1992; other avian examples in Evans, 1987). Morphometric traits are studied as they are directly related to fitness and therefore relevant to conservation (see chapters 5 and 6).

To assess the suitability of protein electrophoresis, the aim was to develop isozyme systems for barn owl blood samples (chapter 7), and then to compare levels of isozyme variation in a sample of captive populations with control groups in the form of self sustaining wild populations- two populations in south west Scotland were selected as suitable for this study (see Taylor et al., 1992; Taylor, 1994). The genetic basis of protein variation can be inferred from pedigree data, and so sampling complete families was desirable; to achieve this aim, a trap was devised, and modified nest boxes were constructed and installed to facilitate the capture of wild breeding adults (Chapter 3). Due to possible sexual dimorphism in isozyme expression or in quantitative traits, a method of identifying the sex of both adults and juveniles was desirable (chapter 4). To assess the suitability of quantitative traits for population genetic monitoring, the aim was to obtain data on external morphological traits which are commonly measured in wild birds, selecting traits which are easily measured (low measurement error) whilst minimising the handling time of the birds. These data could then be used to estimate heritability (chapters 5 and 6). The extent to which these aims were achieved, and the merits of the two approaches to monitoring genetic variation, are discussed in the final chapter (chapter 8).

SUMMARY

Barn owls in Britain have been experiencing a widespread decline since the 1930s. The possible genetic consequences are an increase in levels of inbreeding and a loss of genetic variation. This would contribute to a decrease in their ability to adapt to a changing environment, and may result in inbreeding depression. Accepting the maintenance of levels of genetic variation as the genetic goal of population management, a method for monitoring populations for a loss of genetic diversity would be a useful population management tool. Of a range of available methods for assaying genetic variation, protein electrophoresis and quantitative traits were chosen in this study, to assess their suitability for monitoring genetic change in barn owl populations.

Chapter 2

BARN OWL POPULATIONS

2.1 Introduction

In chapter 1, I stated that a decline in wild barn owl numbers in Britain had prompted concern for its genetic consequences: a declining population may suffer a loss of genetic variability which would reduce its potential to adapt to a changing environment, and a severe reduction in numbers may result in inbreeding depression. It was noted, however, that species differ in the level of genetic variability that corresponds to homeostasis, and that some populations may experience high levels of inbreeding without suffering from inbreeding depression. This inconsistency was used to support the case for developing methods to directly monitor genetic variation, and it was stressed that comparison should be made with a viable (control) population or to monitor a population for change over a number of generations. Even in the absence of adequate controls, however, a step towards the interpretation of levels of genetic variation may be taken, if data are available on a population's past and present demography. This approach has been taken in a number of studies (see Avise, 1994 for review), and is particularly relevant to studies of rare and endangered populations, where no suitable populations may be available to provide reference values for viable populations. In this chapter, therefore, I review what insights into barn owl genetic population management may be gained from demographic evidence rather than by direct monitoring of genetic variation.

The aim is first to provide information on population structure which would be informative in interpretation of data obtained by direct monitoring, and to determine whether initial concerns over genetic issues are justified by the available evidence. Is there evidence to suggest that barn owls naturally experience relatively high levels of inbreeding, for instance, or that they would have low equilibrium levels of genetic variation?

Quantifying levels of inbreeding from pedigree data would be an ideal complementary approach to monitoring genetic variation in isozymes or quantitative traits. Pedigree data were collected by I.R. Taylor for the main wild barn owl population in this study since 1979, but these data were not made available to me for analysis and unfortunately have not been published to date. Inferences about levels of inbreeding and genetic population structure may be made from demographic data, however, and patterns of fecundity, mortality and dispersal, as well as long term population changes, are therefore reviewed in section 2.2.

In section 2.3, some of the genetic issues of population management are discussed; supposing that a loss of genetic variation in wild barn owl populations is considered a problem to be addressed by population management, what are the possible genetic consequences of the population management techniques outlined in chapter 1? To address this question, the barn owl in Britain is first placed in its global context through a comparison with other species and sub-species (section 2.3:1), to give a broad idea of the range of morphological and behavioural differences in barn owls throughout their distribution. This is relevant for management options which involve the translocation of stock, as the question of uniqueness is crucial in conservation biology in determining at what level of organisation conservation effort should be focused (Avise, 1994). If too large a unit is chosen, large scale translocations may result in the loss of locally adapted populations (Taberlet and Bouvet, 1994), or at its extreme, in hybridisation of distinct groups; if too small a unit is chosen, there may be insufficient variation naturally present to allow future adaptation. The possible genetic consequences of translocation and other population management options are summarised in section 2.3:2; finally, in section 2.4, the management implications of the demographic evidence presented in this chapter are reviewed.

2.2: Demographic evidence to aid interpretation of genetic data

2.2:1 Fecundity

Barn owls may breed when in their first year, and in Taylor's study (Taylor, 1994), no significant difference in the number of eggs produced was found between first year and older females. First year birds did tend to lay later in the season than older birds, however, and as first years tended to pair together, this may have been due to the males' hunting proficiency in courtship feeding. Although a large proportion of the breeding population consisted of first year birds in good vole years, few attempted to breed at other times, and so the likelihood of a bird breeding in its first year is dependent on prey abundance. Overall productivity in the population was closely linked with prey availability, with the number of breeding attempts, clutch size and survival of young all varying according to the three year vole cycle, and according to habitat quality within the study area. Although no difference was observed among habitat types in vole decline years, in good years the productivity of pairs in young forestry plantation was higher than in the lowland farms, hence the amplitude of the productivity cycle was larger in the forest habitat. In good years (habitat types combined), a clutch size averaging 5-6 eggs was laid, compared to 3-4 eggs in decline years, egg production being determined by the condition of the female prior to breeding. 5-10% of hatched young died in the nest in peak vole years, with mortality usually confined to the youngest in a brood. In decline vole years, chick mortality was as high as 45%. Annual productivity, defined as the

mean number of offspring per pair reared to fledging, was 4-5 in good vole years, and 1.5- 2.5 in decline years (Taylor, 1994). Occasionally, pairs would rear a second brood in a good vole year, increasing the overall productivity of the population in these years.

Taylor and Massheder (1992) modelled barn owl population change based on the data from Taylor's study. Their model suggests that population growth occurred where the mean annual productivity exceeded 3.2 young per pair. The number of offspring produced per pair in a poor vole year may therefore be lower than would be required to maintain their population at a steady level, yet in good years barn owls have the potential to produce a surplus of young. It follows that areas of particularly good habitat may act as overall 'producers', with the surplus young birds constantly colonising sub-optimal habitat.

2.2:2 Dispersal

There are two methods employed to quantify natal to breeding site dispersal in barn owls. In intensive population studies birds ringed as chicks may be caught as breeding adults in subsequent years. The other method involves ringing returns from adults ringed as chicks, with data drawn from all over the country. Table 2.1 compares the dispersal distances calculated by these two methods for barn owls in Britain; data are from:

(i) BTO ringing returns of birds ringed as nestlings and recovered more than one year later, as reported in Bunn et al., 1982 table 33.
(ii) Natal to breeding site dispersal of barn owls ringed as nestlings and retrapped as breeding adults 1980-1991, in Taylor's study of a barn owl population in Scotland, numbers derived from Taylor, 1994 fig. 13.2.

Table 2.1 Natal to breeding site dispersal distances for barn owls in Britain, comparing data from a national ringing survey with a long term population study

	Bunn et al,. 1982		Taylor, 1994	
Distance from natal site (km)	Number of BTO ring recoveries	% of BTO ring recoveries	Number of retrapped breeding adults	% of retrapped breeding adults
0-5	98	53.30	29	34.94
6-10			38 67	45.78 80.72
11-15	68	37.00	12	14.46
16-20			3 16	3.62 19.28
21-30			1	1.20
31-40			0	0.00
41-50			0	0.00
51-100	9	4.90	0	0.00
101-200	8 18	4.30 9.70	0	0.00
201-300	1	0.50	0	0.00
Total:	184	100.00	83	100.00

Some interesting differences are apparent between the dispersal distances calculated by the two methods. Although both report the majority of birds dispersing less than 50km, the ringing returns show 9.7% of birds dispersing farther, with one individual being found more than 200km from its natal site. No individuals were recorded as breeding farther than 30km in the Scottish population, which showed a large percentage of birds not moving more than 10km to their breeding site: 80.7% compared to 53.3% with the ringing recoveries. Only 19.3% of birds in the Scottish population were found more than 10km from their natal site, compared to 46.7% of the ringing recoveries. Overall, therefore, dispersal distances from ringing returns are greater than those from the population study.

Two explanations have been offered to account for this difference in terms of an overestimation of true natal to breeding site dispersal distances from ringing returns. Firstly, ringing return data include no direct evidence that an adult was occupying its breeding home range when it died. Although strong breeding site loyalty in barn owls suggests that a breeding adult is likely to die within its normal home range, the proportion of unpaired adults in a population has never been accurately quantified, and how the movements of these birds may differ from those of paired individuals is not known. The ability of some populations to expand rapidly when the number of nest sites is artificially increased (Taylor et al., 1992)

suggests that some areas may support a large non-breeding population. If unpaired individuals were more mobile than breeding pairs, possibly dispersing longer distances in search of a breeding site and mate, their inclusion would overestimate natal to breeding site dispersal distances based on adult ringing recoveries.

Taylor (1994) offers a second explanation for the discrepancy; he notes that a large proportion of the mortality reported for ring recoveries is due to collisions with road vehicles or trains, and suggests that dispersal distances may therefore be inflated due to birds becoming stuck to the vehicles that kill them, and being transported long distances before falling off. Taylor supported this theory with evidence from a comparative study of dispersal distances for barn owl road kills and other causes of mortality; 23 roadkills had a dispersal distance of 44.2km, compared to 9.1km for birds which died of other causes (Taylor, 1994 chapter 13).

An intensive local population study which retraps breeding adults has the advantage over recovery data in that there is no doubt that the distances represent natal to breeding site dispersal. This approach may underestimate dispersal distances, however, as long dispersal distances will remain undetected if the bird disperses out of the area of study. A contributing factor to the short dispersal distances recorded by Taylor (1994) could therefore be the unquantified dispersal of some barn owls to breed in farmland adjacent to the study area.

Barrowclough (1978) reviews the problem of underestimating longer dispersal distances in local population studies, and offers a mathematical correction for dispersal distances according to the distance from each natal site to the centre, assuming a circular study area. Correction of dispersal distances is possible even when the study area is not circular, based on the probability of a bird falling within the study area after dispersing a given distance in a random direction from the natal site. This is illustrated in fig. 2.1, where the boundaries of dispersal distance classes from a nest site approximately 10 km from the boundary of a study area measuring 40 * 40km are shown as concentric rings around the site. It can be seen that all the birds dispersing up to 10 km in distance would be detected within the boundaries of the study, but as dispersal distance increases, an increasing proportion of the area into which birds may disperse lies outside the study area. The position of each natal site in relation to the study site boundaries therefore becomes relevant, with more central sites more likely to yield longer dispersal distances than sites close to the boundaries. If concentric rings for dispersal distances are constructed for each natal site, the proportion of each dispersal distance area which is contained within the study area can be calculated. The observed number of individuals dispersing into this area could therefore be adjusted according to the probability of detecting dispersal of that distance within the study area. This would be reasonable where an

appreciable area of the dispersal ring fell within the study area, but would not be appropriate for much longer distances where only a very small proportion of the dispersal ring falls within the boundaries of the study area. Further adjustments could be made to this method, according to the relative suitability of habitat outside the study area; if unsuitable areas were included on the map, the possible dispersal area for each distance could exclude these regions from the estimation. An alternative approach to adjusting the bias in dispersal distances would involve assessing the number of possible nest sites in a given area, rather than excluding unlikely areas. If, rather than calculating the area within the concentric rings of dispersal classes, the total number of potential nest sites is counted, a similar adjustment to the observed numbers of birds could be made, according to the proportions of sites within and outside the study area. If this was attempted for barn owls in a region where buildings were the common nest sites, the relevant area outside the study area could be assessed simply by reference to the appropriate 1:50 000 OS map.

In summary, ringing recoveries may overestimate, and local population studies may underestimate natal to breeding site dispersal distances in barn owls. The general picture remains that most barn owls in Britain breed within 50 km of their natal site, but the possibility of a small proportion of individuals dispersing much further to breed can not be discounted.

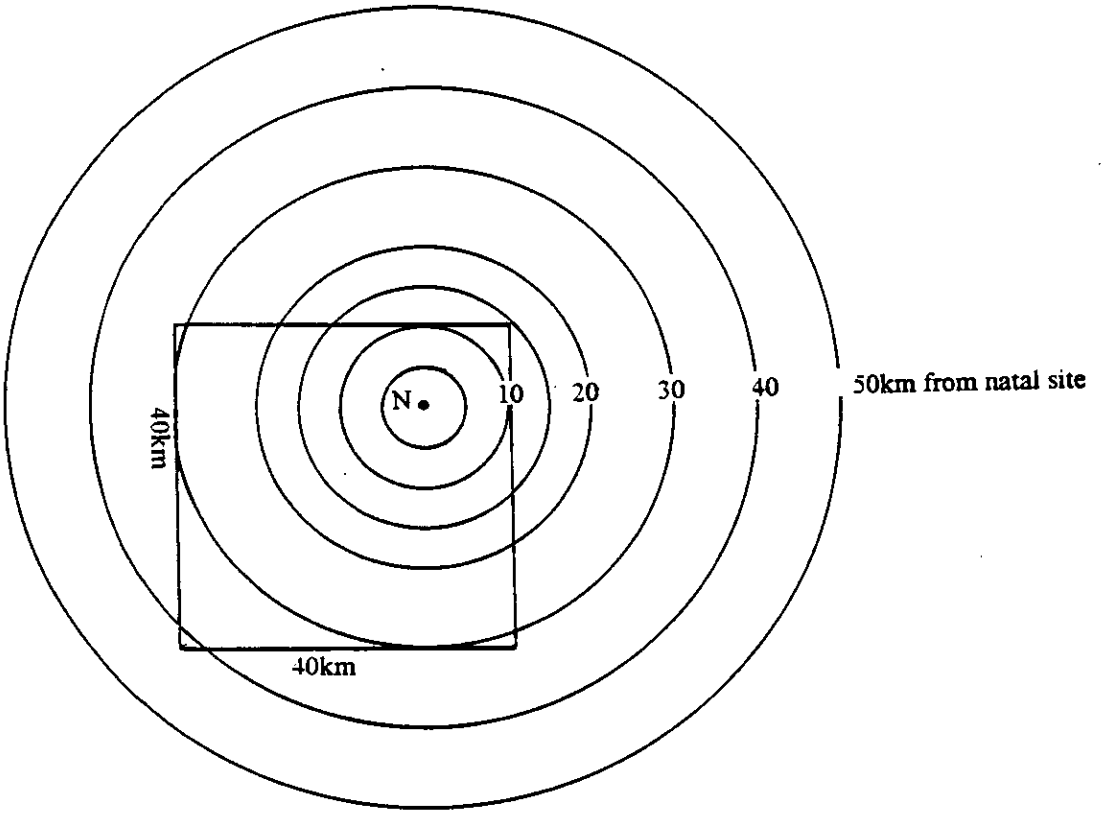


Fig. 2.1 Diagram illustrating how natal to breeding site dispersal distances may be underestimated in a local study. The boundaries of dispersal distance classes from a nest site (N) 10 km from the edge of a study area measuring 40 * 40km are shown as concentric rings around the site. It can be seen that all the birds dispersing up to 10 km in distance would be detected within the boundaries of the study, but as dispersal distance increases, an increasing proportion of the area into which birds may disperse lies outside the study area.

2.2:3 Mortality

The average life expectancy of fledged wild barn owls is estimated at 1.3 years in Europe, yet occasional records demonstrate that barn owls have the potential to live for over 30 years. In temperate climates, first year mortality rates, as calculated from ring recoveries, are in the region of 65-75%; second year mortality is at 40-60%, and third year mortality is at 30-40%; above this, sample sizes become too small for analysis, as only 6-12% of fledged barn owls live to be older than three years (Taylor, 1994).

Taylor (1994 chapter 14) found that mortality rates were highest during the winter in the Esk population, for both first year and older birds, and also were strongly cyclical, correlating with winter vole availability. An annual adult mortality of around 30-50% was recorded in years of low vole abundance, whereas in good vole years, adult mortality did not exceed 30%.

Ringing returns in Britain showed that road and rail fatalities were the most common recorded causes of barn owl deaths, with the proportions of first year and older birds remaining constant over an 18 year period (1970-1988) at 49% for first years and 48% for older birds. (Percival, 1990).

Shawyer (1987) analysed 629 records of adult barn owl mortality in Britain for the period 1982-1986, data being drawn from reports from the public. The results for England, Wales and Scotland were: road deaths 51.9%; drowning 6.2%; collision with wires 5.1%; trapped in buildings 3.1%; shooting and trapping 2.7%; poisoning 3.2%; predation 2.4%; rail deaths 1.7%; aircraft deaths 0.5%, and 23.2% with no apparent cause, although starvation was thought likely in many cases.

Of 627 barn owls sent for autopsy during the period 1963-1989, Newton et al. (1991) recorded traffic collisions and starvation as the main causes of death, with poisoning by organochlorines (aldrin/dieldrin) accounting for 40% of the carcasses from the south-east of Britain up to 1977.

Biases in the methodology leave these results open to debate, however. A major problem involves the confusion of proximate and other causes of death; for example, in Victoria, Australia, cestode parasites were found infesting the gizzards of barn owls which died of starvation (Mcorist, 1989); sub-lethal levels of the rodenticides difenacoum and brodifacoum were found in 10% of barn owls in Britain whose deaths were attributed to other causes (Newton et al., 1990), and many reported road casualties are in an emaciated condition. The extent to which the less apparent stresses influence mortality is not known, but they may be

the determining factors which eventually lead to death. Some road casualties, for instance, may be due to reduced vigilance or behavioural changes such as use of sub-optimal habitat, or dispersal induced by starvation.

In addition, the chance of carcasses being found is not random with respect to cause of mortality, with a bias in favour of the more conspicuous deaths such as road kills. This was highlighted by an intensive study by Taylor (1994), in which 138 first year birds and 66 older birds were recovered over a six year period. Locals in the study area in SW. Scotland were encouraged to report barn owl carcasses; possible roost and nest sites, and roads, were searched monthly. The results revealed a difference in mortality causes for first year and adult barn owls; 56.5% of the first year birds were road casualties, compared to 22.7% of adult fatalities; 33.3% of first year deaths were due to starvation at roost sites, whereas 65.2% of adult deaths were due to roost site starvation. A direct comparison of this method of assessing mortality and that of ringing returns was not possible, as it is not known what proportion of these birds would have been found and reported in the absence of intensive searching. The results contrast with the national figures based on ringing returns, however, suggesting that a high proportion of adult mortality usually remains undetected, with starvation being underestimated, and that road kills account for a greater proportion of first year than adult fatalities.

It may be concluded from these surveys that mortality is highest for barn owls in their first year, with traffic collisions being a major cause of death at the time the young birds are dispersing.

2.2:4 Long term population changes

Monitoring barn owl populations is not a simple task; being largely nocturnal and often nesting in remote places they may easily remain undetected unless an intensive search is made for them. Add to this the cyclical pattern of breeding according to vole availability, and it becomes clear that only an intensive and long term study is likely to give an accurate estimate of population numbers. Such studies must be constrained by time and resources over the area which can be reliably surveyed, yet barn owls in different parts of the country may be experiencing quite different ecological conditions, and populations may not be following the same trends throughout the country. The total population of barn owls in Britain today has therefore not been accurately determined, although some attempts to come up with a population figure for Britain have been attempted. A census in 1932 based on a questionnaire for members of the public combined with surveys by local naturalists estimated the population in England and Wales at 12 000 pairs. An estimate of 1 001 to 10 000 pairs was

suggested in 1973 from a historical review of breeding birds in Britain. A survey for The Atlas of Breeding Birds in Britain from 1968- 1972 resulted in an estimate of 4 500 to 9 000 pairs in Britain and Ireland, based on 2-4 pairs occurring in each 10km square in the country in which barn owls were reported. A survey covering the period 1982-1985 in which members of the public were encouraged to report known barn owl nests, and incorporating detailed fieldwork in some areas, resulted in an estimated 3 750 pairs in England and Wales, and 650 pairs in Scotland (Shawyer, 1987). A review of population estimates for the period 1982-1985 placed the current British population at around 4400 breeding pairs (Gibbons et al., 1993).

Although methodological problems exist in all these surveys, some generalisations may be made concerning population trends in Britain, based on the surveys, and supported by perceived trends by local farmers and naturalists. Changing patterns of land use and farming practices allow speculation on longer term population changes giving an approximate description of barn owl population changes in Britain from the time the country was first occupied by barn owls.

The last glacial period in Britain ended abruptly around 10 000 years before present (bp); initially tundra type vegetation predominated, but forests spread as the climate warmed. Yalden (1982) estimates that all the native British mammals were present by 9 500 bp, before the English channel flooded around 9 000 bp. Fossil evidence of barn owls has been found in caves in England dating to around 10 000 bp (Shawyer, 1987), suggesting that they were fast to arrive in the country following the retreat of glaciation. Preying on lemmings and other small rodents, the barn owl population may have been limited by suitable nest sites at this time, confined to regions where there were caves and cliff holes. By around 7 500 bp, most of Britain was covered with forest, which suggests that barn owls were not common at this time due to a lack of suitable habitat. As humans began to open up the forests for agriculture, both nest sites and suitable habitat would become available for the barn owls, allowing a rapid expansion and increase in numbers, and there was probably a long period during which barn owls were abundant in Britain. There is certainly evidence that barn owls thrived in the farming landscape before the 1930s; they were a common sight around the small mixed farms typical of this time. From the 1940s, farming practices began to change rapidly. Fertilisers and pesticides resulted in continuous use of land instead of the practice of leaving fields fallow; machinery replaced horses and so field size increased for ease of management, and grazing land for horses was no longer required; many other changes also occurred (see Taylor, 1994 chapter 16) resulting in an environment less suitable for barn owls, including a loss of nest sites due to changes in farm buildings and a loss of old trees. Pesticides such as dieldrin may have contributed to the further decline in barn owls in some parts of the country in the

1950s and 1960s, and the second generation rodenticides which were widely used from the 1970s are also fatal for barn owls if even a small number of poisoned prey are consumed (Taylor, 1994 chapter 16).

In Britain, therefore, barn owls probably existed in relatively low numbers until forests were cleared for agriculture, and they were at their most abundant when agriculture was based on small mixed farms. Since the 1930s a widespread decline began, which accelerated in the 1960s and 1970s. Causes of the decline include a loss of habitat and nest sites, and the use of pesticides. The available evidence suggests that a similar decline is occurring in much of the European range of *T. a. alba,* although it is still considered common in some countries (e.g. Portugal; Shawyer, 1987).

2.2:5 Conclusions: Demographic evidence to aid interpretation of genetic data

Lande and Barrowclough (1987) calculated that neutral variation would take 10^5 to 10^7 generations, at a large effective population size ($N_e = 10^5$ to 10^6) to regain former levels of heterozygosity after most was lost by drift in a population bottleneck, whereas heritable quantitative variation would regenerate in populations of around $N_e = 500$, in 10^2 to 10^3 generations. Single-locus deleterious recessive alleles, by comparison, could recover in around 10^2 generations. If barn owls in Britain are assumed to have experienced a bottleneck at the time of colonisation after the last ice age, current levels of isozyme variation would not be expected to have recovered to pre-bottleneck levels in the subsequent 10 000 years, yet appreciable amounts of other genetic variation, such as heritable morphometric variation could have accumulated in that time.

A low equilibrium level of neutral variation may be maintained even in the absence of severe bottlenecks; low levels of isozyme variation have been correlated with a polygynous mating system, large and highly mobile species, dietary specialists and fragmented populations (Negro and Hiraldo, 1994), and therefore may be predicted for a specialist predator such as the barn owl. Low levels of isozyme variation in the bald eagle *Haliaeetus leucocephalus* (Morizot et al., 1985), the cape griffon vulture *Gyps coprotheres* (van Wyk et al., 1992), and the total lack of isozyme variation in the Spanish Imperial eagle *Aquila adalberti* (Negro and Hiraldo, 1994) indicate that a general background of low isozyme variation may be common amongst raptors, although data from Peregrine falcons *Falco peregrinus* (Morizot, 1988) demonstrate that this is not universally the case.

A low predicted level of isozyme variation in barn owls is not indicative of immunity to the deleterious effects of inbreeding, however, as if the population bottleneck occurred in the

distant past, deleterious recessive alleles could have accumulated since then through mutation. An initial examination of the dispersal distance data for wild barn owls suggests that relatively high levels of inbreeding would be tolerated in viable wild populations; 80.7% of barn owls in Taylor's study bred within 10 km of their natal site, for instance (Taylor, 1994). Taylor found 1.2% of his birds dispersing 20-30km to breed, however, and ringing return data suggest some barn owls disperse over far greater distances. Given that the exchange among sub-populations of a very few individuals per generation can counter the effects of inbreeding, and that close inbreeding may be avoided due to female siblings dispersing further on average, than males (Taylor, 1994), the available data do not support the theory that wild barn owls naturally experience high levels of inbreeding. There is therefore a risk of inbreeding depression if levels of inbreeding in barn owl populations were to increase.

Modern patterns of fecundity, dispersal and mortality suggest that barn owls in Britain today exist as a 'meta-population' where gene flow occurs more readily within certain areas than between them, but with few, if any, populations in complete isolation. Patterns of gene flow would be complicated, depending on the costs of dispersal among populations, with areas of good habitat acting as overall sources of young birds; sub-optimal habitats acting as 'sinks'. As habitat deteriorates in quality due to changing agricultural practices, fewer surplus young capable of colonising new areas would be produced; as habitat becomes more fragmented, the risk of mortality during natal to breeding site dispersal would increase, particularly where busy roads separate pockets of suitable habitat. Compared to the situation around 70 years ago, when farming practices provided ideal barn owl habitat, populations may now occur at lower densities, and also be increasingly isolated. Initial concerns over the genetic consequences of a population decline in barn owls would therefore seem justified on the basis of the demographic data.

2.3: Genetic aspects of population management

This section addresses the question of determining the appropriate 'units' for conservation of barn owls; at what level of population organisation should conservation effort be focused? This issue is crucial in conservation management, in influencing how resources are partitioned, and in determining whether populations should be treated as separate entities, or whether the translocation of stock derived from different geographical areas should be permitted. The uniqueness of barn owls as a taxon is first described in 2.3:1; differences among species and sub-species are then described in more detail. The genetic consequences of management options are described in 2.3:2.

2.3:1 Variation among species and sub-species

(a) Taxonomy and evolution

The earliest fossil record of an owl (*Ogygoptynx wetmorei*) is a tarsometatarsus from the Paleocene (70-60 million years ago, mya) in Colorado, thought to be an intermediate form of the two modern owl families, the Tytonidae (barn and bay owls) and the Strigidae (all other owls) (Olson, 1985). Numerous other fossil remains have been found; Eocene (60-40 mya) owls from North America belong to the extinct family Prostrigidae which are quite different to either the Tytonidae or the Strigidae. A coracoid from the Miocene (25-12 mya) in France may represent the earliest example of the modern genus *Strix*; of the Tytonidae, *Prosybris antiqua*, also from the early Miocene, may be the earliest recorded specimen. Three species of *Tyto* are recorded from the middle to late Eocene in France, and two additional species in Italy. Of these, *T. robusta* and *T. gigantea* were much larger than modern Tytonids. Three *Tyto* species are known from the Quaternary (2-3 mya to present) in Cuba, *T. alba, T. noeli and T. riveroi*, which differ greatly in size. Very large barn owls have also been recorded from the Quaternary (*T. ostologa* in Hispaniola; *T. pollens* in the Bahamas) and from the Plio-Pleistocene (*T. balearica* from Majorca and Minorca). (Olsen, 1985). Remains of *T. balearica*, possibly predating the type specimen, have also been recorded in the Pliocene (12-2 mya) in Spain and southern France (Mourer-Chauvire and Sanchez-Marco, 1988).

The division between the Strigidae and Tytonidae in the fossil record is based on the osteological differences of modern owls. These are illustrated by radiographs of the barn owl *Tyto alba*, and the greater horned owl *Bubo virginianus* by Smith and Smith (1991). The great horned owl, for example, possesses a humeroscapular bone which is entirely absent in the barn owl (Smith and Smith, 1992).

Further evidence for owl taxonomy comes from DNA-DNA hybridisation data, karyological studies and protein electrophoresis (reviewed in Randi et al., 1991). The cladistic approach by Cracraft (1981) placed owls as a superfamily of Falconiformes, whereas DNA-DNA hybridisation data led Sibley et al. (1988) to conclude that Falconiformes and Strigiformes were only distantly related. Evidence from the electrophoresis of egg white proteins, and from mitochondria (mMDH) indicate an affinity between Strigiformes and Caprimulgiformes (oilbirds, potoos, nightjars and nighthawks), and Randi et al. (1991) take this as a working hypothesis, using a 'caprimulgiform' for their outgroup comparisons of protein variation within the Strigiformes.

Both DNA-DNA hybridisation data and egg white protein electrophoresis suggest a large difference between *Tyto* and the Strigidae. This is supported by karyological evidence, as the

diploid number, 2n is 92 in *Tyto*, but averages at 82 in Strigidae; there are also structural differences in the chromosomes of these two groups, as in *Tyto*, all the chromosomes are acrocentric, and there is no abrupt distinction between macro and micro-chromosomes. Randi et al. (1991) calculate a Nei's average genetic distance, based on protein electrophoresis, between Tyto and Strigidae of D=1.49, and suggest a separation time for the Tytonidae and Strigidae of more than 40 mya, and also calculate that the modern Strigidae evolved not earlier than the lower Miocene, which is supported by the fossil evidence.

It appears, therefore, that barn owls evolved as a group quite distinct from all other owls, and were widespread early in their evolution; they exist in modern times as a 'sister lineage' of the Strigidae.

(b) Modern distribution of barn owls

Within the present day Tytonidae, two species are classed in the genus *Phodilus;* they are the Oriental bay owl *P. badius*, and the African bay owl *P. prigoginei.* Nine species are recognised in the genus *Tyto* (barn owls), and the divergence in morphology and ecology has allowed the occupation of a wide range of habitats in different parts of the world. The larger species may attain weights of 1000g or more, for instance the masked owl *T. novaehollandiae* and the sooty owl *Tyto tenebricosa* of Australia and New Guinea, which prey on large marsupials (Taylor, 1994 chapter 2). The other species are *T. longimembris, T. multipunctata, T. rosenbergii, T. inexpecta, T. aurantia, T. soumagnei* and *T. alba,* which between them occupy habitat ranging from open grassland to closed forest, with only areas of extreme cold, deserts or remote islands remaining devoid of barn owls.

Tyto alba alone has a total of 35 or 36 distinct geographical forms; their global distribution is illustrated in fig. 2.2. Many of the sub-species are unique to small islands, yet others such as *T. a. affinis* in Africa south of the Sahara desert, *T. a. delictula* in Australia and the Solomon Isles, and *T. a. pratincola* in North and Central America, are very widely distributed. Native British barn owls are of the *T. alba alba* subspecies, which is found in the UK, Ireland, Channel Islands, Spain, Portugal, west and south France, Italy, former Yugoslavia, Greece, and North Africa, and they form a hybrid zone with *T. a. guttata* in eastern France and western Germany. (Taylor, 1994 chapter 2). Barn owls come close to the most northerly limits of their range in Britain.

Fig. 2.2: Map showing the global distribution of *Tyto alba* sub-species. The distribution of *T. a. alba* is shaded in black.

Reproduced from Taylor, 1994, fig. 2.1

Distribution of barn owl subspecies. 36 subspecies have been described, although some confusion exists over the status of some. Precise details of distribution are uncertain for many and the map is intended as a general guide. 1. *T. a. alba* (Scopoli): UK, Ireland, Channel Is., Spain, Portugal, west and south France, Italy, Yugoslavia, Greece, N. Africa. 2. *T. a. guttata* (Brehm): Denmark, Netherlands, Belgium, Germany, eastern Europe. Hybrid zone with *alba* in eastern France/western Germany. 3. *T. a. schmitzi* (Hartert): Madeira. 4. *T. a. gracilirostris* (Hartert): Canary Is. 5. *T. a cinesti* (Kleinschmidt): Corsica, Sardinia. 6. *T. a. detorta* (Hartert): Cape Verde Is. 7. *T. a. affinis* (Blyth): Africa, south of Sahara. 8. *T. a. thomensis* (Hartlaub): Sao Thome'. 9. *T. a. hypermetra* (Grote): Comoros Is., Malagasi. 10. *T. a. erlangeri* (Sclater): Saudi Arabia, Oman, Gulf states north to Lebanon, Syria, Iraq, Iran. 11. *T. a. stertens* (Hartert): India, Pakistan, Bangladesh, Sri Lanka, Assam, Sikkim, Nepal, Bhutan, Burma. 12. *T. a. javanica* (Gmelin): Thailand, Burma, Indo-China, Malaysia, Indonesia, Java, Flores, Timor. 13. *T. a. descopstorfii* (Hume): Andaman Is. 14. *T. a. sumbaensis* (Hartert): Sumbe Is. 15. *T. a. everetti* (Hartert): Savu Is. 16. *T. a. kuehni* (Hartert): Lesser Sunda Is., Flores to Timor; confusion possible with distribution of *javanica* and *everetti*. 17. *T. a. meeki* (Rothschild and Hartert): south east New Guinea, Vulcan and Dampier Is. 18. *T. a. delicatula* (Gould): Australia, Solomon Is. 19. *T. a. crassirostris* (Mayr): Boang Is., Tanga Group, Bismark Archipelago. 20. *T. a. interposita* (Mayr): Santa Cruz Is., Banks Is., northern New Hebrides. 21. *T. a. lulu* (Peale): New Caledonia, south New Hebrides, Fiji, Loyalty, Tonga, Samoa, Society Is. 22. *T. a. pratincola* (Bonaparte): North and Central America. 23. *T. a. guatemalae* (Ridgeway): Panama to Guatemala. 24. *T. a. lucayana* (Riley): Bahama Is. 25. *T. a. furcata* (Temminck): Cuba. 26. *T. a. niveicauda* (Parkes and Phillips): Is. of Pines, Cuba. 27. *T. a. bondi* (Parkes and Phillips): Bay Is. (off Honduras). 28. *T. a. glaucops* (Kaup): Tortuga and Hispaniola, West Indies. 29. *T. a. nigrescens* (Lawrence): Dominica, West Indies. 30. *T. a. insularis* (Pelzeln): Lesser Antilles. 31. *T. a. bargeri* (Hartert): Curacao Is. (off Venezuela). 32. *T. a. contempta* (Hartert): Columbia, Ecuador, Peru, Venezuela. 33. *T. a. subandea* (Kelso): parts of Columbia, Ecuador. 34. *T. a. hellmayri* (Griscom and Greenway): Guianas to Amazon. 35. *T. a. tuidara* (Gray): Brazil (south of Amazon), Chile, Argentina. 36. *T. a. punctatissima* (Gray): Galapagos Is.

Differences in plumage among sub-species

Plumage coloration varies markedly among sub-species. The palest is *T. a. alba*, where males are often completely white on their underparts and pale buff above, with little flecking or mottling; females are typically more heavily marked, with black flecks on their underparts, a richer buff colour above, and more pronounced wing bars, flecks and mottling. Some of the darker sub-species have little or no white plumage, with buff coloration extending over their underparts, extensive and dark flecking and streaking, larger wing bars and some greyish plumage. Darker forms are found on small tropical or sub-tropical islands, and in forested habitats, whereas the paler forms are more typical of open grassland.

Differences in body size among sub-species

Taylor (1994, chapter 2) reviews morphometric data for 22 subspecies of *T. alba*; *T. a. javanica* of south-east Asia were the heaviest, with a mean male weight of 555g, whilst *T. a. punctatissima* of the Galapagos Isles was the lightest, with a mean female weight of 264g, compared with 303g for *T. a. alba.* The linear measurements of tarsus, wing and tail lengths did not always vary proportionately within the sub-species; *T. a delicatula* of Australia, for example had tarsi 23% greater than *T. a. alba*, although its wing length and body weight were less (Taylor, 1994). Barn owls in south-east Asia and some of the South American sub-species had proportionately shorter wings, and island forms were smaller overall than the mainland sub-species.

Dietary differences among sub-species

Barn owls regurgitate the indigestible remains of their prey as pellets, which can be found at nest and roost sites. As the prey is usually swallowed whole, the identification of skulls in the pellets provides a quantitative measure of the prey consumed. Photographs of barn owls bringing prey to the nest, and an examination of the prey items cached at nest sites confirms that pellet analysis is a reliable measure of the prey taken (Taylor, 1994). A large number of studies throughout the world have investigated aspects of barn owl diet in this way, and have provided data on the prey populations, in some cases revealing prey species which had not been detected locally by other methods (Purger, 1990).

Taylor (1994) reviews 54 of these studies, selected to give a world-wide picture of barn owl diet using long term studies on populations of several nest or roost sites where possible. Diet was analysed in terms of prey numbers and biomass. Small mammals accounted for the majority of the prey taken by barn owls; in 33 of the studies, rodents made up more than 75% of the prey items.

In most of temperate Europe and North America, voles are the dominant prey species; On the continent, and in Orkney, it is the common vole *Microtus arvalis* whereas in mainland Britain, where the common vole is absent, the field vole *Microtus agrestis* is the main species. Shrews are usually the secondary prey species, but are occasionally the dominant species.

In Mediterranean regions, much of the tropics and sub-tropics, Australia and on many islands, rats and mice are the most important prey. The number of prey species on which barn owls feed varies according to their abundance; where small mammals are abundant, barn owls tend to specialise in a small number of species, for example, in Europe, 3-4 species make up 80% of all prey items taken. Where small mammal density is lower, a wider range of species is preyed upon, hence in Africa 5-6 species make up 80% of prey taken (Taylor, 1994). Species which contribute occasionally to the barn owls diet include bats (e.g. nine species of bat found in pellets in Poland, Lesinski, 1989); birds (22 species in pellets in Germany, Jentzsch, 1988); amphibians, insects, and even fish (Taylor, 1994).

The prey that is actually taken depends on a number of factors. There is some variation in the potential prey species that can be handled by the barn owl; for instance, a laboratory study on *T. alba guttata* prey size preferences revealed an upper limit of 80g where the prey offered were active rats and mice of a range of sizes, with 10-40g being the size range most frequently selected (Ille, 1991). As the sub-species of barn owl differ in size, their dietary requirements and upper prey size limits also vary.

The habitat determines the prey species which are present, but barn owls do not simply feed on the prey according to their abundance. Behavioural and life-history characteristics of the prey affect their vulnerability to predation, also the barn owls preference for more profitable (i.e. larger) catches. Derting and Cranford (1989) noted that male *Microtus pennsylvanicus* and *Peromyscus leucopus* were caught more often than the females, and attributed this to behavioural differences of the prey. Activity may often be more important than size of prey; Dickman et al. (1991), for instance, found that young female house mice *Mus domesticus* were captured more frequently than their abundance would suggest; fluorescent marking revealed that they used open vegetation areas more frequently than the adults, due partly to adult interference.

The factors which influence prey selection do not remain constant over time, and this is reflected in changes in diet both seasonally, and over longer time periods. Seasonal variation is seen in most areas of the barn owls range; voles are less prominent in the diet during the months of March to May in Scottish barn owls, when shrews dominate the diet (Taylor,

1994); in South Africa, *Mastomys natalensis* and *Mus minutoides* dominated the diet towards the end of winter, whereas *Otomys irroratus* and *O. saundersae* were the summer prey (Wirminghaus, 1989). In North America, pocket mice and gophers were preyed upon in summer, but were unavailable in winter, as the mice hibernate and the gophers remain in winter burrows; insects formed up to 80% of prey items caught in Sicily, during the two month dry period (Taylor, 1994).

Longer term changes in diet are seen where the main prey species change in abundance. In Europe, voles appear to undergo population fluctuations on a 3-4 year cycle throughout the barn owls' range, and although they may be the main prey in years of high abundance, barn owls are forced to rely on suboptimal species when the vole populations crash. A similar pattern is seen with the rodent *Notomys alexis*, which is subject to large fluctuations in population size in the Tanami desert, Australia (Smith and Cole, 1989).

Differences among sub-species in other aspects of their ecology

As may be expected, barn owls which live in different geographical areas in quite different habitats and with different prey species, also differ in most other aspects of their lives. Data from ringing returns, for instance, suggest that British barn owls have shorter dispersal distances than European birds. 90% of British barn owls were found within 50km of their natal site, and 53% were within 10km. This compares with figures from Germany, where 30% went further than 100km; Dutch and French recoveries showed intermediate values (Taylor, 1994). In North America, 80% of southern birds were recovered within 80km of their natal site, whereas in northern states, 31% were found further than 100 miles away. Other behavioural differences are seen in the selection of nest sites, with British birds accepting sites close to the ground, which would be unacceptable to barn owls in regions where mammalian nest predators occur (Taylor, 1994). Other varying traits include clutch size, the timing of breeding, and longevity.

Taylor (1994) explains the morphological variation between sub-species in terms of adaptive significance, with differences in body size and plumage related to ecological differences in habitat use and prey types: arboreal sub-species have shorter wings, and sub-species which hunt over long grass have longer legs. Island forms were consistently smaller than the mainland sub-species, which Taylor relates to their diet, as insects are often important prey on the islands. Darker coloured arboreal sub-species and paler plains dwellers may have the adaptive advantage of being harder to detect by potential prey or by predators in each habitat. As most of the islands where barn owl sub-species are found would originally have been forested, the darkness of plumage of the island forms may fit this generalisation.

(c) Implications for management

This review shows that barn owls are a distinct group of owls with an almost global distribution of species and sub-species; they differ in body dimensions, plumage coloration, and many aspects of their ecology. Assuming a heritable basis to these traits, and that different selection pressures have been in operation in different environments, it may be concluded that significant genetic differences distinguish the sub-species. A strong case is therefore presented for conserving *T. a. alba* as a discrete entity, and that population management options that allowed sub-species to interbreed should not be permitted.

Whether gene exchange among different geographical areas should be permitted or even encouraged within the *T. a. alba* subspecies is open to debate; differences among populations would be expected to be less severe than those recorded among sub-species, and no study has addressed population differentiation in terms of ecology, morphology or genetic markers in any detail in *T. a. alba*. A case for maintaining uniqueness could be argued against mixing stock derived from populations which would not naturally experience gene exchange; major barriers to gene flow within *T. a. alba* are suggested by the distribution map (fig. 2.2), where sea divides the sub-species into North African, European, Mainland Britain and Irish populations. Dispersal between these areas is likely to be much less frequent than dispersal among populations within each region, although occasional reports of continental birds recovered in England indicate that the barrier to gene flow may not be total.

2.3:2 Genetic consequences of population management options

The population management options listed in chapter 1 as possible methods of encouraging self-sustaining barn owl populations were: providing artificial nest sites, habitat improvements, supplementary feeding, translocating wild individuals, release of captive bred individuals. In this section, the possible genetic impact of these options is considered.

(a) Artificial nest sites and habitat improvements

Providing artificial nest sites may be all that is required to increase the wild breeding population; if habitat is adequate, nest sites may be the limiting factor for population expansion, and there may be a pool of non-breeding birds which could rapidly become established as breeders if sites were available. This was demonstrated in a study of barn owls in SW Scotland, where nest boxes and plastic drums in trees at the edges of plantations were provided at 57 sites. The number of sites occupied increased from 3 to 31 over four years (1985-1988) (Taylor et al., 1992). Improvement of habitat (with or without artificial nest sites, as appropriate) would have a similar effect on population size by increasing productivity

and density of the wild population. The genetic impact of these strategies would be similar; by increasing the population size, loss of genetic variability through drift would be reduced, whilst no new genetic material is introduced to the remaining wild population. They differ in the speed with which population expansion is likely to occur, however; providing nest sites in areas of good habitat already occupied by barn owls would rapidly increase the breeding population and so minimise the effects of drift; habitat improvements may be expected to occur more gradually.

(b) Supplementary feeding

This option involves providing food at roost or nest sites, for example dead day old chicks from the poultry industry. Providing food over winter would reduce adult mortality, and encourage birds to enter the new season in good condition for breeding; productivity may therefore be increased. Feeding may be necessary throughout the breeding season to ensure high offspring survival. By improving adult survival and increasing productivity, this strategy increases the wild population size, but this is only a useful option if there are areas of suitable habitat capable of supporting the artificially increased population. The genetic impact is similar to that described above, as a rapid increase in numbers is possible. How this strategy may differ from the provisioning of artificial nest sites or broad habitat improvements is that only those birds nesting or roosting at the target sites are favoured, presuming that it is not feasible to locate and provision all the potential breeding sites in a population. By increasing the productivity of only a proportion of the remaining wild birds, the variance in family size is increased. The competitive advantage given to families reared at the favoured sites may result in the loss of other potential recruits to the breeding population; a loss of genetic variability in the population as a whole despite an increase in numbers is therefore the risk of this strategy.

(c) Translocation

Translocation from other wild populations may redress the effects of inbreeding in the target population by introducing new genetic material, and may reduce the loss of genetic variation in the recipient population by increasing their opportunity to breed and by increasing population size.

If the differences between donor and recipient populations are large, however, genetic problems are possible. If the divergent populations have adapted to local conditions, or have been separate for so many generations that co-adapted gene complexes have evolved in the different groups, mixing the populations may result in a loss of fitness in the offspring, described by Templeton (1986) as outbreeding depression. Even apparently beneficial results of a translocation may not be sustainable in subsequent generations; the mixing of two inbred

populations may initially result in an increase in fitness in the progeny (hybrid vigour) but the advantages may be lost in subsequent generations.

In an extreme example of the impact of translocations, the resultant hybrids between introduced and native stock may differ ecologically from the native population, possibly with undesirable consequences. When the American cordgrass *Spartina alterniflora* was introduced to Britain, for example, it hybridised with the native *S. maritima* to produce *S. anglica*, which proved to be more vigorous than the native cordgrass. (Green, 1979).

Where the desires to maintain genetically distinct populations, and to avoid the extinction of small populations are in conflict, perhaps because there are no thriving populations of a particular genetically distinct group, a compromise must be found. The brown bear *Ursus arctos* in Europe is an example of a such a compromise. In this case, as all the populations of the Iberian refugium are endangered, Taberlet and Bouvet (1994) recommend that the Iberian and Balkan refugia be thought of as a single conservation unit, allowing translocation of individuals from the less endangered Balkan populations to augment the Pyrenean population.

(d) Captive breeding and release

Captive breeding may result in severe genetic divergence of the captive stock from the wild populations; the major problems concern founder effects and inbreeding, as captive populations must usually be based on a small number of individuals derived from the wild. Temple and Cade (1988) describe three potential bottlenecks in a raptor recovery programme that involves captive breeding; they are:

- A sample of birds is taken from the wild to captivity.
- A sample of their offspring is released.
- A sample of released birds become established as breeding birds in the wild.

In each case, the manager may reduce the founder effects, by initially selecting a diverse set of individuals, avoiding matings between close relatives, equalising the contributions of the founders, and expanding numbers rapidly to minimise the loss of alleles.

If the initial breeding stock is obtained from populations other than the one targeted for the release of the progeny, new alleles may be introduced to the wild population, and the same considerations described above for translocations apply. Additional problems may occur if the captive stock is maintained in captivity over many generations; apart from possible inbreeding, selection may inadvertently occur in favour of individuals suited to the captive environment, a process counter-productive for a successful release scheme.

Selecting breeding stock from the area where it is intended to release the progeny avoids the introduction of new genetic material into the wild population, and not breeding from captive bred birds avoids the problems of inbreeding in small captive populations, but even a captive breeding programme managed in this way may still have an impact on the genetic structure of the wild population. The captive parents are likely to produce more offspring than their wild conspecifics, particularly in a species such as the barn owl, where reproductive output is linked to the food supply (Taylor, 1994, 11.1), and where the life expectancy of captive stock may far exceed that of wild breeding birds. Increasing the variance in family size in this way may actually reduce the genetically effective population size of the wild population, increasing inbreeding by the influx of a large number of closely related individuals (Ryman and Laikre, 1991). The outcome depends on the balance between the beneficial effect of increasing the overall population size, against the detrimental effects of a disproportionate representation of certain genotypes.

This is an extension of the established idea that the genetic contributions of the founders of a small captive population should be equalised to maximise the effective population size; in this case, equalising genetic contributions would involve taking the entire remaining wild population into captivity, a practice which is rarely possible or desirable.

2.4: Management implications

What can be concluded about barn owl population management on the basis of this review of barn owl demography and the genetic consequences of various management options?

Firstly, the population decline in barn owls in Britain is a cause for concern. Habitat deterioration and loss results in increasingly fragmented areas of suitable habitat; a combination of lower productivity in poor habitat, and increased costs of dispersal among habitat patches, results in increasing isolation of the remaining populations. A loss of genetic variability is predicted due to the decline in numbers, and there is the risk of inbreeding problems if populations become small and isolated. Theory based on mutation rates and the effects of drift suggests that populations with an effective population size of several hundred (500 or more) individuals would be desirable to maintain genetic variation in quantitative traits. Although this should not be accepted as a firm rule, populations of 10s rather than 100s of individuals must be considered at risk. Barn owls typically have short natal to breeding site dispersal distances, but whether the less frequent dispersal of individuals for greater distances provides sufficient gene exchange among sub-populations to counteract the effects of drift is not known. If the decline continues, it may be predicted that this becomes increasingly a problem for population management.

Of the management options described here, habitat improvements and the provision of additional nest sites are the options calculated to have the least genetic impact, and both are necessary in the long term, if self sustaining wild populations are to be maintained; their loss is identified as the main cause of the decline in the first place. Other strategies may be appropriate to minimise the loss of genetic variation through a rapid increase in numbers; supplementary feeding is likely to be effective, as barn owl productivity is closely tied to food availability. Translocations of wild individuals may be recommended if wild populations are small and isolated; birds from adjacent sub-populations may be the best choice, whereas barn owls of other sub-species should not be used due to differences in ecology and morphology. A case could also be argued for confining the choice to examples of *T. a. alba* drawn from the same landmass as the recipient population, as the sea is considered a significant barrier to gene flow. *T. a. alba* from Africa or the continent should not be translocated to Britain if other stock are available, for instance. The release of captive bred barn owls is an option which could, if properly executed, result in the rapid establishment of a breeding population, but there are more genetic problems associated with this option than any other. Ideally, a large number of breeding birds would be available, drawn from wild stock either from the area of intended release, or from diverse parts of the country, depending on the desired impact of the release on the remaining wild population; birds intended for release should not be the progeny of many generations of captive breeding. Suitable breeding stock is readily available in the form of road casualties, the majority of which are young dispersing birds, likely to be a random sample of the wild population. It is therefore suggested that these birds should be the basis of any such breeding programme.

Although predictions concerning the genetic impact of different management options are valuable in aiding the preliminary development of a management strategy, the patterns of genetic change may be imperfectly predicted in this way. As described in chapter 1, monitoring changes in genetic structure directly is therefore desirable in population management, and this is the subject of the following chapters in this thesis.

SUMMARY

Modern patterns of fecundity, dispersal and mortality suggest that barn owls in Britain today exist as a 'meta-population' where gene flow occurs more readily within certain areas than between them, but few, if any, populations exist in complete isolation. Compared to the situation around 70 years ago, when farming practices provided ideal barn owl habilat, populations may now occur at lower densities, and also be increasingly isolated. Initial concerns over the genetic consequences of a population decline would therefore seem justified on the basis of the demographic data.

The eight modern species of barn owls have an almost global distribution; among the 36 forms of *Tyto alba* both morphological and ecological differences have been described, and so it is recommended that population management options that allow sub-species to interbreed should not be permited. The possible genetic consequences of habitat improvements, artificial nest sites, supplementary feeding, translocation and captive breeding are outlined in the final section.

Chapter 3

POPULATION SAMPLING PROCEDURES

3.1 Introduction

This chapter describes how data were collected for investigating variation in isozymes and quantitative traits in wild and captive barn owl populations The sampling aims are outlined in section 3.2, and details of the populations studied are described in section 3.3. The methods used to catch the birds, and a description of the procedure for processing each bird are presented in section 3.4. Section 3.5 concludes by assessing how closely the achieved sampling matched the original aims.

3.2: Sampling aims

(a) Isozymes

The most convenient source of varying proteins for a study of this nature is a blood sample from each individual. All sampling would ideally occur from birds of the same age, stage of breeding cycle, and during the same season, to reduce errors introduced by temporal and environmental changes. Isozyme frequencies may differ among age groups, for instance, which Evans (1987) attributes to selection, dispersal or because a population does not have a stable age distribution, but could alternatively be due to differences in expression of the isozymes in each age group.

The detection of polymorphic loci from isozyme frequencies is strongly dependent on sample size. A locus is usually considered polymorphic when the most common allele has a frequency of less than 0.99, although some studies define polymorphism as a frequency less than 0.95 (Evans, 1987). An allele frequency of 0.01 could not be detected if the sample size was less than 50 individuals, as the lowest frequency represents a single individual heterozygous for the rare allele; an allele frequency of 0.05 would require a minimum sample size of 10 individuals. Much larger sample sizes are desirable, however, if an allele of low frequency is to have a high probability of detection; the probability of detecting an allele occuring at 5% frequency in a sample of 10 individuals, for example, is $1 - 0.95^{20} = 0.64$, and around 30 individuals would be required to detect the allele with a probability of 0.95. A sample size of 10 individuals may therefore be taken as the absolute minimum if isozyme data are to be analysed in the conventional manner, but larger samples are recommended if possible, particularly if sexual dimorphism in the isozymes studied may occur. Ideally, the heritable basis of the observed variation would be tested using data from parents and offffspring, and so a population sample much larger than the minimum of 10 individuals would be required.

(b) Quantitative traits

Quantitative traits conveniently measured on live birds in the field include weight, wing and tarsus length. As with the isozyme study, data from parents and offspring are desirable to allow inheritance of the traits to be investigated, in this case by estimating heritability (see chapter 6). Offspring should ideally be sampled when fully grown to allow direct comparison of parental and offspring traits. A large sample of birds is desirable; the merits of sampling a few offspring from many parents, or of maximising family size, are covered by Falconer (1981), where the design of breeding experiments for estimating heritability is discussed. Quantitative traits are even more likely to be influenced by temporal and environmental changes than are isozyme frequencies (chapter 6), and so unavoidable confounding effects, such as the age of the birds when sampled, should be recorded where possible.

Outline of sampling aims

In a study operating under financial and temporal constraints, the choice has to be made between sampling a small number of populations in great detail, or obtaining data from more populations at the expense of the sample size per population. In any case, populations available for study may have fewer individuals than would be acceptable if the study concerned the genetics of a species which could be manipulated under controlled conditions. The sampling aim then becomes to sample as many individuals as are available in the allocated time, where in some cases, the 'sample' may represent the entire population.

My aim was to obtain data from a minimum of 10 barn owls from each of several captive or wild populations, and to concentrate sampling effort on one wild population to catch complete families of barn owls, sampling offspring at the nest when they had completed skeletal growth and were close to fledging. To this end, nest boxes were constructed and installed, and birds were caught during the breeding season with a hand held net and with a box trap used in conjunction with a nest box. Care was taken to ensure that the procedures had no detrimental effects on the birds; Taylor (1991) had shown that repeated nest visits to barn owls in the Langholm population (popn. 1) made no difference to the number of young fledged at the sites visited, or to the weights of the young at fledging, when compared to sites which were visited only once when the young were about to fledge.

From each bird, a blood sample was taken for isozyme analysis (chapter 7), and to measure the percentage of red blood cells in the blood, which may be an indication of the birds condition (chapter 5). Weight, both tarsus lengths and both wing lengths were recorded for an investigation of heritability of these traits in the main wild population (chapter 6), and to look

at possible asymmetry in the birds (chapter 5). Plumage was photographed to provide data on sexual dimorphism (chapter 4); in addition, pin feathers were taken from some young birds for karyological sex determination (chapter 4).

Each bird was assigned a sex and age where possible; this was known in many cases where breeding adults had been ringed as pulli. Age of unknown adults was estimated according to the moult pattern of primary feathers, as described by Taylor (1994, chapter 8). Unknown sex birds were sexed according to their plumage (see chapter 4).

3.3 Populations

In this study, a wild population consisted of breeding pairs, their offspring, and some non breeding individuals which inhabited the same geographical area. A captive population consisted of all the captive barn owls held at one location, or at several locations in the same area, some of which were breeding stock, and some were young birds intended for release.

Five populations were selected for study, representing two wild and three captive populations which were likely to yield a minimum of 10 birds per population. One wild population was sampled in more detail in an attempt to sample as many entire families of barn owls as were available in the three years of study.

The two wild populations were both the subject of long term population studies, which was a high priority in their selection due to the considerable benefits of working with established study sites- all breeding sites in the populations had been located, and permission to visit the area was obtained from landowners; the majority of the birds were individually identifiable as they had been fitted with BTO rings, and pedigree data had been collected over a number of years. Although the pedigree data collected prior to this study were not made available to me, pedigree data were collected over the three years of this study. In both populations my sampling was assisted by the people most familiar with the barn owl populations in their study area.

Sites were visited under licence from the Nature Conservancy Council (NCC) and Scottish Natural Heritage (SNH) and blood sampling was carried out under Home Office licence.

As far as could be told from ringing data, no captive reared barn owls had been released in the vicinity of the two wild populations, and there was no evidence of direct dispersal between the two wild populations, although neither was isolated and immigrants may have entered the study areas. The small number of unringed birds appearing in the study areas as adults may

represent immigrants or birds which fledged from inaccessible sites within the study areas (dispersal is discussed in chapter 2).

Details of the five populations are summarised below, and the locations of populations 1 and 2 are shown on a map of south Scotland (fig. 3.1)

Population 1

The main wild population had been the subject of a population study by Dr I. R. Taylor (Edinburgh University), since 1979. In the south west of Scotland, approximately 70 km from Edinburgh, it consisted of the catchment area of the river Esk in Dumfries, covering 1600 km^2, from coastal plains up to a height of about 500m. At lower altitudes, fields were maintained for cattle or sheep grazing and for hay and silage production; small woodlands covered about 20% of the area. Above about 150m, the landuse was low density sheep grazing and plantation forestry, hence a range of habitat types were available to the barn owls. This study area is described in detail by Taylor (1994, chapter 1).

Nest sites included abandoned cottages, barns and haysheds, with the nests under the eaves, on upper floors, down chimneys or between hay bales. Many sites had wooden nest boxes, which prolonged the time that a decaying building would be a suitable nest site, and also made certain modern buildings available. Approximately 50 such sites were used by barn owls for breeding or roosting in the three years of this study, with the greatest number of breeding pairs occurring in 1990, a year of high vole abundance in this area, when 30 pairs of barn owls were located.

Fig. 3.2 illustrates the rough grassland habitat in the Langholm study area, with an abandoned cottage used as a nest site by barn owls; fig. 3.3 shows one of the wooden nest boxes used by barn owls in this study area (see also fig. 3.7).

Population 2

This wild population had been monitored since 1979 by G. Shaw and A. Dowell (Forestry Commission). West of population 1, it covered the Cree valley north and west of Newton Stewart, Galloway. In this area, rough grazing and hill land had been extensively forested with Sitka spruce *Picea sitchensis* and Norway spruce *Picea abies*, and a mosaic of plantations from recently planted to mature trees was present. Barn owls nested mainly in abandoned buildings until 1985- 1988, when the available nest sites were increased by the provision of nest boxes and plastic drums in trees at the edges of plantations at 57 sites, where rough

grassland provided suitable habitat in forest rides, river edges and in young plantations. This study area is described in Taylor et al., (1992).

Fig. 3.4 illustrates the habitat in this area, in this case, where broad strips of grassland border a road. Fig. 3.5 shows one of the modified plastic drums used as nest sites in this study area.

Population 3

The Barn Owl Trust, Ashburton, Devon kept three breeding pairs of barn owls as permanent breeding stock, whose offspring were released in Devon. The breeding birds consisted of one pair of wild, disabled birds from Cornwall, a wild Devon road casualty paired with a female bred at the Trust from parents of unknown captive bred origin, and a male of unknown captive bred origin paired with a daughter of the second pair described above. Other birds at the Trust included other wild, disabled birds and some captive bred individuals. Barn owls at the Trust were sampled in December 1990, and October 1991. In addition, birds at a further six locations in Devon and Cornwall were sampled in October 1991; they were mainly in pairs kept for breeding. These were in the charge of individuals who had been invited to cooperate with my study by the Barn Owl Trust.

Population 4

The South Midlands Breed and Release Scheme breeds barn owls for release in Oxfordshire, Buckinghamshire, Northamptonshire and Warwickshire. Breeding stock are either donated by the RSPCA and bird hospitals, or are purchased from other captive breeders; stock is chosen where possible from different parts of England. Birds intended for release as adults are reared in breeding aviaries, then overwintered together in a large (120' * 24' * 10') holding aviary, which may accommodate around 50 barn owls. These birds were sampled in December 1990 and September 1991.

Population 5

A small population of barn owls was maintained at the Falconry Centre, Hagley, west of Birmingham. Of a total population of 18 birds, 12 were made available to me for sampling at the time of one visit in October 1992. Although detailed pedigree records were not available for this population, individuals of a sub-species other than *T. alba alba* had been bred with *T. alba alba* at the centre, and some birds had been released locally.

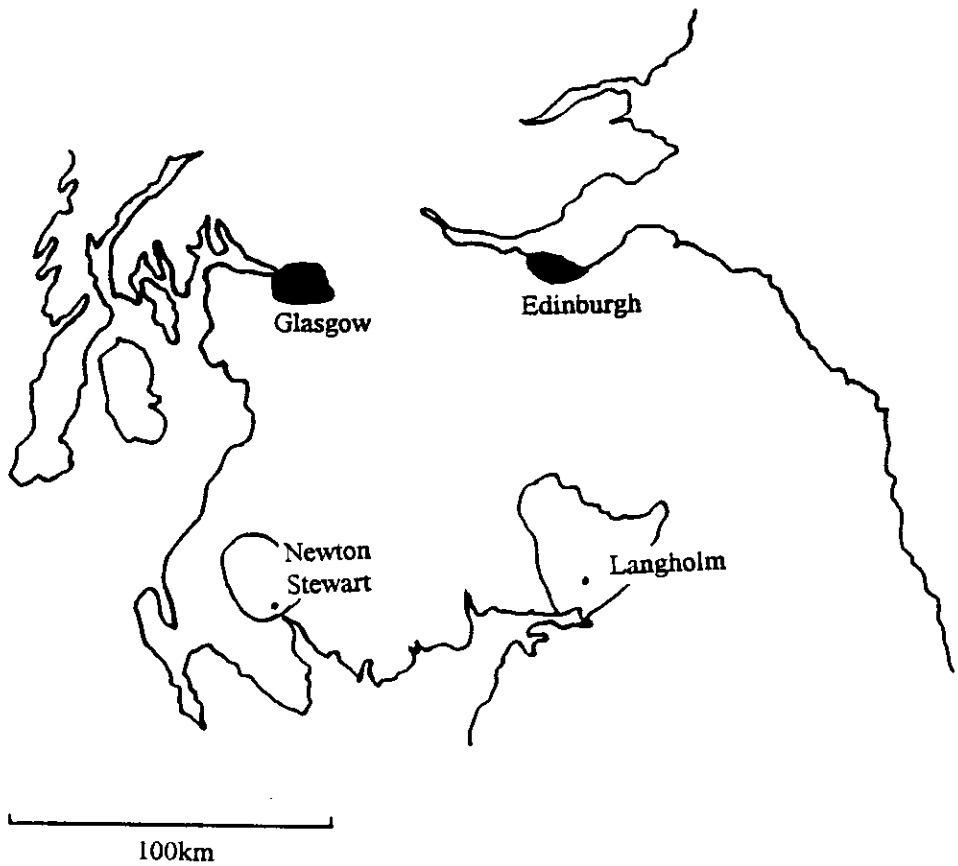


Fig. 3.1 Map of south Scotland showing the locations of the Langholm (population 1) and Newton Stewart (population 2) study areas

Fig. 3.4 (above) Habitat in the Newton Stewart study area: wide strips of rough grassland border a road.

Fig. 3.5 (right) A plastic drum nest box in the Newton Stewart study area, put up to test the hypothesis that nest site availability limited barn owl numbers in this area (see Taylor, Dowell and Shaw, 1992)

Fig. 3.2 An abandoned cottage used as a nest site by barn owls in the Langholm study area. Rough grassland and plantation edges provide good foraging habitat around this site.

Fig. 3.3 One of 30 nest boxes installed in buildings in the Langholm study area during the winter of 1991 / 1992. A trap designed to catch adult barn owls when they enter to feed their young is shown here with the door closed, in the tunnel leading to the nest cavity. (See also fig. 3.7)

3.4 Methods employed to catch and sample barn owls

3.4:1 *Timing of Sampling*

(a) Captive barn owls

Captive barn owls were sampled at the convenience of their keepers; this was usually during the winter when the birds were not breeding or nearing the time for their release, and all samples were obtained in one or two visits to each group. Catching these birds in their aviaries was a relatively simple procedure, and was usually carried out by their keeper, allowing the sampling to proceed in rapid succession. A maximum of 20 birds were sampled in one day.

(b) Wild populations

Catching the wild birds was more complicated, repeated visits to a nest site being necessary to assess breeding progress and to enable the capture of both parents and their offspring. The optimum time to catch breeding pairs was at the beginning of the breeding season; male and female typically roost together during pairing and egg laying, with the male less likely to be on site as incubation proceeds. At this time, attempts were made to catch both adults at a site by the use of a hand held net, as detailed in section 3.4:2. The relatively short time that males were on site, coupled with their greater wariness of the site being approached, resulted in a sampling bias in favour of females, which could be caught at any time until they stopped brooding the chicks when the youngest was 12-16 days old (Taylor, 1994 chapter 12). In the Langholm area it was possible to modify some sites to create a further opportunity to catch the adults by means of a nestbox trap. This involved trapping the parents at night, when they attempted to enter their nest box to feed the young. The design and use of the trap are described in section 3.4:3.

Juveniles were sampled at two stages of development; feather samples for chromosome analysis were taken when they were approximately 35 days old, whereas the blood samples, measurements and photographs were taken as close to fledging time as possible, at 50-60 days old. Ideally, juveniles would have been sampled at precisely the same age, but this was not possible for a number of reasons. Firstly, direct evidence of the hatching date was only rarely available, from the few occasions when a nest inspection coincided with an egg in the process of hatching (two occasions during the three years of study). Taylor inferred hatching date from a wing length growth curve (Taylor, 1994) which introduces obvious problems of circularity for a morphometric study. In addition, as hatching occurs asynchronously in barn owls, with siblings 1-2 days apart in age, nests visits to sample the offspring at precisely the same age would have to occur at 1-2 day intervals over the period spanning the ideal sampling age for

each chick. This intensity of site visits is greater than the frequency determined by Taylor (1991) to be safe in welfare terms; its impact is not known. The repeated site visits required to sample offspring at precisely the same age would also have considerably increased the time and resources required for data collection, from one visit per site to up to seven visits, depending on family size; this would not have been feasible without compromising the total number of birds sampled. The nature of the site also influenced the age at which the juveniles could be sampled; birds reared in large nest boxes or in buildings could be sampled at a later stage than those which were reared in the forestry plantation barrels; they would typically leave the barrel when nearing fledging, and roost in the dense conifers nearby.

3.4:2 Use of nets to catch adults

(a) *Net specifications*

The net used most frequently had a circular frame of 1 metre diameter. At some sites, a similar net of smaller diameter was more appropriate.

The net frame was constructed from a flexible plastic curtain rail. A discarded fishing net with a 1" mesh was used for netting; being well worn, it was soft and pliable. The bag of the net was deep, allowing it to be closed in the manner of a butterfly net, by folding it over the frame. A cord attached to the bottom of the bag allowed the net to be held open when approaching a bird, facilitating its passage to the very bottom of the bag with least resistance. The net could be extended by the use of 5' sections of aluminium tubing which screwed together securely; most sites were accessible using one or two of these poles.

(b) *Procedure at the site*

Where possible, the nest box or barrel was approached quietly with the net extended, until the net was positioned over the entrance hole, as illustrated in fig. 3.6. If no adults emerged, the net was held in place while an assistant positioned ladders, climbed up and blocked the entrance hole. The box could then be inspected. Birds would occasionally attempt to remain in the box, typically incubating females or birds habituated to this method of capture. In this case they could be carefully picked up by hand. Gloves were rarely used due to the loss of dexterity and the risk of damaging plumage. Pairs could be caught in one netting attempt; if the first bird to enter the net appeared to be a male, the net was held in place until another bird followed or the entrance hole could be blocked. Once in the net, it was folded over to close the bag, and lowered to the ground where the birds could be picked out. If more than one adult was caught at one time, they could be retained in cotton bird bags until sampling was complete. Birds caught in this way were returned to the box after sampling, and the hole

blocked for up to a minute to encourage them to stay in. All equipment (ladders, etc.) was removed from the site, as quickly and quietly as possible.

When nest boxes were in inaccessible parts of buildings, or where the birds nested on bare rafters, in the eaves or down a chimney or tree hole, the nets were used to cover the most likely route of departure. These were identified from past experience at a particular site, or by the presence of barn owl droppings at a window, hole, or skylight. An assistant would then approach the nest site from the opposite direction, flushing the birds towards the net. After sampling, these birds were released within the building, after removing all sampling equipment from the site.

Fig. 3.6 Using a hand held net to catch adult barn owls from a nest box. The net is positioned over the entrance hole, and by holding out the cord attached to the bottom of the pocket, the bird is directed into the deepest part of the net. This nest box is a wooden A-frame design, in the Newton Stewart study area.

3.4:3 Use of nestbox-traps to catch adults

(a) *Box specifications*

I constructed 30 nest boxes suitable for use with a box trap; they were put up during the winter of 1991 / 1992 to replace existing nest boxes or at sites where no box was present in the Langholm study area.. The boxes (see figs. 3.3 and 3.7; also fig. 16.11 in Taylor, 1994) incorporated a short tunnel leading to the nest cavity. A 25cm drop from the tunnel to the bottom of the box prevented smaller chicks from wandering into the tunnel, and kept it free from the build up of pellets during the nesting period. A platform at the tunnel entrance was included for the benefit of the chicks as they neared fledging. The boxes, made of 1cm ply-wood coated in a non-toxic wood preservative, were constructed as upper and lower parts which were assembled on site; this reduced the weight that had to be carried up a ladder at one time, and made the boxes more manoeuvrable. Wood shavings to a depth of approx. 5cm were put in the box once it was in place, to allow the female to make a 'scrape' to lay in.

(b) *Trap specifications: 1991*

The box trap was made of a 'sleeve' of thin ply-wood, which fitted into the nest box tunnel, flush with the outside rim. The first model had the inner end blocked by 1" wire mesh, painted black to reduce it's visibility from the outside. The vertical sliding door consisted of a black painted steel plate held open by a pin mounted inside the box at an upper corner, and connected to a ply-wood treadle towards the rear of the trap with bicycle brake cable. The steel door was cut away at the bottom, so that it could not trap tail feathers if the closure was activated before the bird was completely inside the trap. The two parts of a magnetic circuit breaker were mounted on the door and on the box, such that the circuit was completed when the door closed. A length of cable led from the trap to a battery powered light, hence it was usually possible to set the trap and wait in a vehicle parked near the site.

(c) Use of trap: 1991

Initial trials in 1991 with the first model of the trap proved unsatisfactory. Although it functioned as planned on its first use, at other sites the approaching adult became wary on seeing the door poised above the tunnel entrance. Cautious entry into the trap did not trigger the door to close, as the mechanism required the full weight of the bird at the far end of the trap. At two sites, where a tunnel had been added to an existing nest box, the alerted adult proceeded to feed the young by squeezing the prey through gaps at the box-tunnel junction, in preference to entering the trap; the young at these sites were old enough to approach the adult and to be passed the prey intact. The use of this trap was discontinued after four trials; in each

case, the trap was removed and 2-3 voles (supplied by my cat) left in the box, as soon as an adult had made one visit to the nest, to compensate for the disruption of a night's hunting.

(d) *Trap specifications: 1992*

In 1992, modifications to the trap resulted in its reliable use for catching adults. The modified trap is illustrated in fig. 3.8. Where the trap was used in non standard nestboxes, care was taken to attach the tunnel leaving no gaps at the sides.

The inner end of the sleeve was covered with fine mesh plastic strawberry netting, which was less visible than the wire mesh used previously. The door was made of light weight, black painted aluminium hinged near the top of the trap; when set, it was out of sight inside the trap, held in place by a pin connected to a thin, dark coloured cord which led towards the back of the trap and crossed the trap at a height of 5cm, 12cm from the far end, where it functioned as a 'trip-wire'. The cord then passed through a hole in the side of the trap, and was held loosely in place with masking tape. This allowed the tension of the cord to be adjusted from the outside, and allowed the cord to become loose as soon as it was triggered. A very small movement of the cord resulted in the door closing. A mechanism for keeping the door closed once it had been triggered was necessary; spring clips were used initially, but required the full weight of the door to swing down unimpeded, as otherwise, the door did not pass them. This resulted in the escape of one bird, where the closing door had apparently been slowed down by brushing over it's back or tail feathers. The use of a magnetic catch, retailed as a catch for cupboard doors, was reliable in keeping the door closed, and required no force from the door to operate.

(e) *Use of trap: 1992*

The trap was set during the evening, (7-9pm.); setting the trap, arranging the wires inconspicuously, removing the ladders and establishing a suitable place to wait took approximately 15 minutes. When the light mounted in the vehicle indicated a capture (9:40pm -12:15am), the owl was removed from the trap immediately, and once sampled, was released outside the nest site, after all equipment had been removed. 2-3 dead voles were left at the box.

Trapping for males took place when possible when the chicks were still being brooded by their mother at around 12-16 days old. The female would then be left inside the box, prevented from leaving by the trap in the tunnel. This had the advantages that the male would be encouraged to enter the box to pass prey to the female, and that there was no risk of the trap being triggered by the female entering with prey.

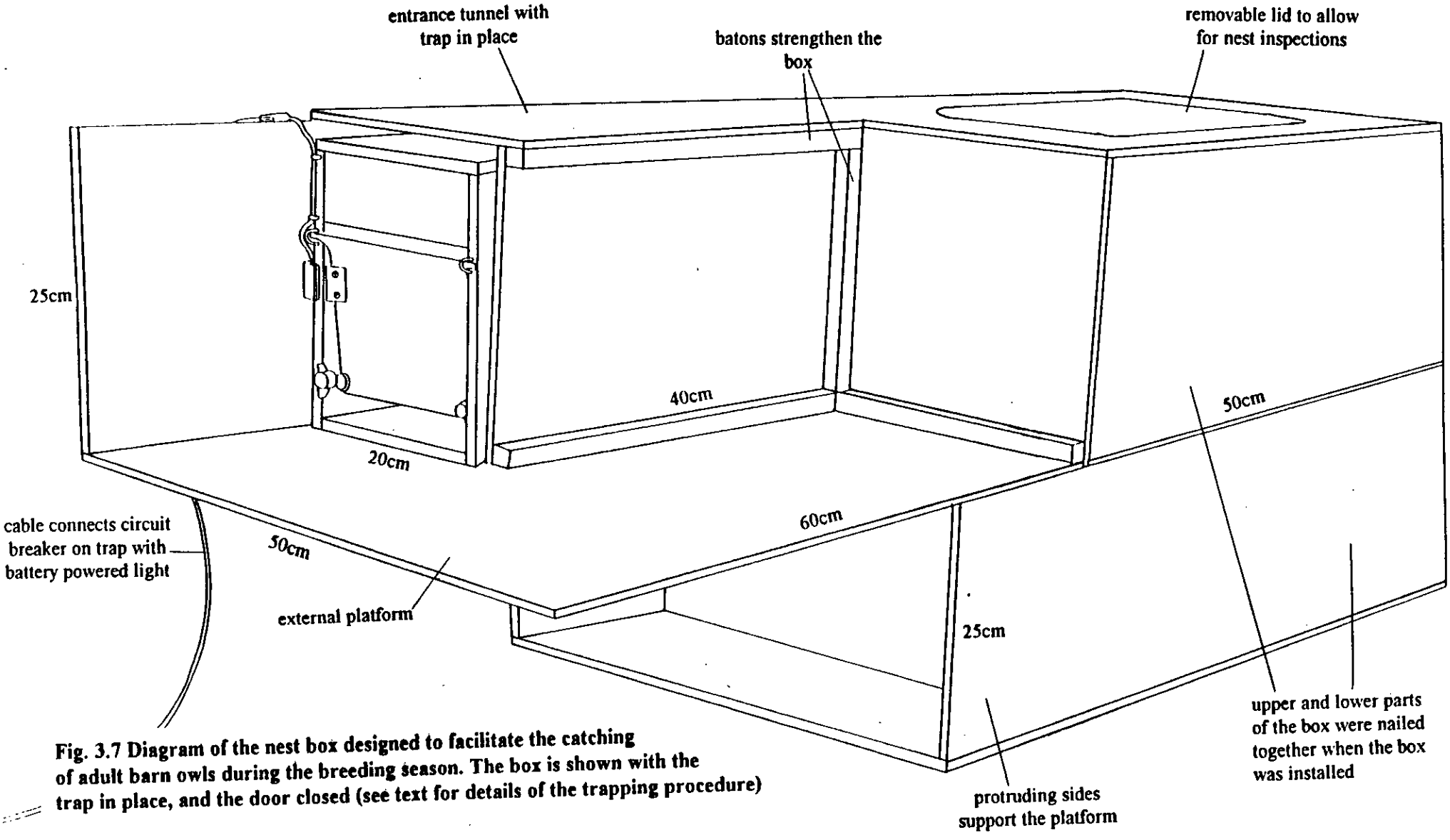


Fig. 3.7 Diagram of the nest box designed to facilitate the catching of adult barn owls during the breeding season. The box is shown with the trap in place, and the door closed (see text for details of the trapping procedure)

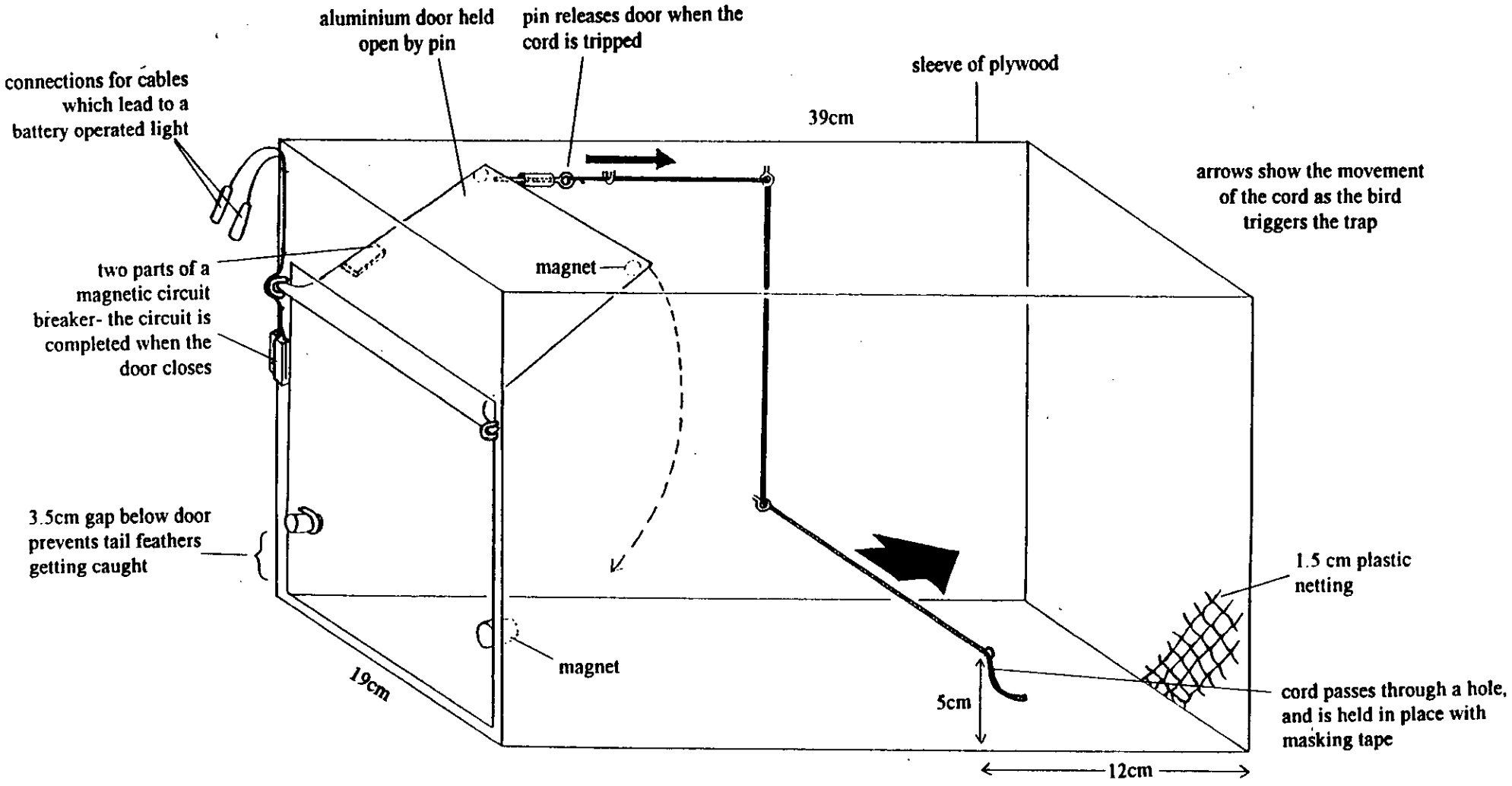


Fig. 3.8 Diagram of the trap used in conjunction with the nest box illustrated in fig.3.7 to catch adult barn owls during the breeding season. The trap is shown here with the door set open (see text for details of the trapping procedure)

At two sites, pairs did not choose the new nest boxes in preference to the place used the previous year, resulting in clutches being laid in close proximity (< 2 metres) to a nest box. In order to trap adults at these sites, the chicks were left until they were no longer being brooded by the female, and although not feathered enough to fly, were mobile within the building. At this stage they were placed in the nest box during the day, and their crops checked the following day to confirm that they had been fed in their new site. At both sites the chicks had been attended by their parents but at one site, the chicks had not remained in the nest box, but had returned to their original corner. At the other, they remained in the box; the male was successfully trapped at this site the following week.

3.4:4 On site procedure

(a) Feather sampling for sex determination (see chapter 4)

This procedure was carried out on a trial sample of 30 barn owls in population 1, when they were around 35 days old. One or two of the larger pin feathers from the upper coverts were grasped firmly at their base, and slowly pulled out in the direction of their growth. No bleeding occurred when these feathers were pulled. The feathers were immediately transferred to collecting tubes as supplied by Databird International, and transported in a cool box. Samples were despatched by courier to Databird within 24 hours of sampling.

(b) Blood sampling and morphometrics

This procedure was carried out for each barn owl caught. Once captured, the sampling procedure was completed in approximately 15 minutes per bird. When several chicks were sampled at one site, siblings were retained in a cardboard box until sampling was complete; adults were secured in bird bags.

Each bird was assigned a sample number on site, and details of sex and age were entered on a reference card. The bird was first weighed in a cotton bird bag with a 500g balance, then left and right tarsi measured using dial callipers accurate to 0.01cm. For ease of measurement, and hence a high repeatability in tarsus measurements (see chapter 5) the measurement of the 'tarsus' was taken from the prominent protuberance of the fibula at the point of articulation with the tarsometatarsus, to the point of articulation of the tarsometatarsus with the first phalanx of the middle toe. The measurements were taken with the bird lying on its back with legs flexed, in the same position as would be used if fitting a ring on the tarsi; the positions of the bones could be felt by moving the legs and feet slightly. Right and left wings (flattened chord) were measured with a 300mm wing-rule accurate to 0.1cm.

Two photographs of the bird were taken using Kodak Ektachrome 200 film, showing dorsal and ventral views of the entire bird with the left wing fully extended, against a white cloth background and including the reference card. The bird was held by an assistant, taking care that the hands obscured a minimum of plumage.

Evans (1987) recommends that 0.12ml of blood per 10g body weight may be taken from a bird without causing harm, and so a 1ml blood sample from a bird weighing over 300g allows a large safety margin. Even so, blood samples were not taken from birds judged to be in poor condition. The assessment was based on the sharpness and depth of the keel when handled, and their general appearance as well as their weight.

Blood samples were taken from the brachial vein in the right wing, with a heparinised 25G sterile needle and syringe. The bird was restrained on its side by an assistant holding the legs, and the right wing fully extended; the head was covered with a bird bag to minimise stress and reduce movement. A foam cushion provided support for this position; this greatly improved the ease of sampling, as the correct position displayed the vein prominently. The area surrounding the vein was swabbed with 70% ethanol; this sterilised the area, exposed the vein by dampening down the surrounding feathers, and increased the blood volume in the vein. The needle was bent at an angle to aid insertion, and inserted into the vein close to, but pointing away from, the body. By this method, blood was diverted from the vein to the needle without disrupting its flow, allowing 1ml to be withdrawn in seconds. Following this, the needle was removed, and gentle pressure applied to the point of insertion for approximately two minutes. An insufficient period of applied pressure resulted in a hematoma; in this case pressure would be resumed until bleeding from the vein had ceased completely. The bird was returned to the nest as soon as sampling was completed.

The blood was transferred directly from the syringe to a 1ml Li-heparinised collecting tube, and swirled gently to ensure the action of the anti-coagulant. The needle was first removed to minimise the number of times the blood passed down its narrow bore, which could cause cell disruption. The needle and syringe were then reunited, labelled and retained, as they contained approximately 40ul blood suitable for DNA analysis.

A small volume of the remaining blood (approximately 70ul) was then drawn by capillary action into a glass hematocrit tube, labelled, and sealed at one end with plasticine.

All blood samples were transported from the site in a cool box, and taken to be processed usually within eight, and always within twelve hours from sampling.

(c) Immediate sample processing in the laboratory

The blood retained in the syringes was transferred by repeated flushing of the barrel into pre-prepared 1ml eppendorph tubes containing 1ml 95% ethanol, and stored at 0-4 °C in the fridge, as this provided a possible source of DNA for future analysis.

Hematocrit tubes were centrifuged at 10 000g for 10 minutes, and the percentage of the red and white cell fractions recorded; the use of hematocrit scores to assess condition is discussed in chapter 5.

The remaining sample was centrifuged at 10 000g for 10 minutes, after which the plasma fraction was pipetted into 1ml eppendorph tubes, and the white layer above the red cell fraction discarded. The red cell fraction was diluted with an equal volume of distilled water, and mixed thoroughly; plasma and red cell fraction tubes were stored in a -20 °C freezer until required for isozyme analysis.

3.5 Results of sampling procedures

(a)Birds sampled

Table 3.1 summarises the number of samples obtained in each population over the three years that data were collected. Birds assigned a sex in this table were of known sex due to breeding records, although many of the birds classed as unknown sex in this table were subsequently assigned a sex on the basis of their plumage (see chapter 4).

Of the birds sampled, only four individuals which were first sampled as pulli in the Langholm population were later resampled as breeding adults. In none of the other populations were individuals sampled both when pulli and when adult. Seven of the breeding adult females in the Langholm population were resampled once in subsequent years; in each case they had remained loyal to the breeding site where they were first sampled. One adult male in this population was sampled twice in the same year, as he was apparently the breeding male at two separate sites.

In the Newton Stewart area, five adult females were sampled twice in different years, and one female was sampled three years running, at the same site each time. Of the captive populations, three birds were sampled twice in population 4, as a complete set of data had not

been attained on the first visit. Repeated samples of the same birds were not included in the analyses, unless otherwise stated in the appropriate sections.

Table 3.1 Table showing the number of barn owls sampled in each population over the three years of data collection.

Population	year	adult male	adult female	unknown sex adult	pulli	total
Population 1	1990	1	8	0	24	33
	1991	13	24	1	23	61
	1992	12	12	3	38	65
	total	26	44	4	85	159
Population 2	1990	6	7	0	0	13
	1991	6	13	1	8	28
	1992	9	14	0	0	23
	total	21	34	1	8	64
Population 3	1990	7	7	0	0	14
	1991	4	6	5	4	19
	1992	0	0	0	0	0
	total	11	13	5	4	33
Population 4	1990	1	5	10	0	16
	1991	3	5	12	0	20
	1992	0	0	0	0	0
	total	4	10	24	0	36
Population 5	1990	0	0	0	0	0
	1991	0	0	0	0	0
	1992	4	4	0	4	12
	total	4	4	0	4	12
Total:						304

In the wild populations, some birds were caught on several occasions, both within a year during site visits to check the progress of the families, and in different years. They were not resampled if blood samples and photographs had already been taken; the figures in table 3.1 therefore under-represent the total number of birds caught each year. These birds are included

in the pedigree records, if they were known to be related to other members of the population. The pedigree data for all populations are presented in appendix 1; it should be noted, however, that an absence of a relationship between any two birds in a population reflects a gap in the available pedigree data, and does not imply the lack of a relationship. The complete pattern of inter-relatedness in a population would only emerge if data were available from many generations, as all individuals would be related to some extent.

(b) Were the sampling aims achieved?

A minimum of 10 birds sampled from each population was achieved in all populations. Data from related birds were obtained from each population; the largest data set is available from population 1, where pedigree data were available from 114 birds, consisting of 30 families with 1-7 offspring. Data from this population were available from both parents for 16 families, from one parent only (3 male, 7 female) for 10 families, and 4 families consisted of siblings only, allowing for the possibility of testing for inheritance in the isozyme data and heritability of morphometric traits. Pedigree data from the other four populations were not extensive enough for heritability estimates; population 2 included data from 2 families consisting of both parents plus offspring; population 3 included 5 sets of siblings with 2-5 individuals per family, population 4 had 1 complete family of both parents and 4 offspring, and 5 sets of siblings (2-3 per family) and population 5 included both parents of 2 families with 1 and 3 offspring. The details of the available pedigree data are shown in appendix 1.

Birds were not all sampled at the same age, season or year; although wild breeding adults were sampled at a consistent time, their offspring were usually sampled just before fledging, at 50-60 days old. Although tarsus extension is completed by around 40 days of age, wing length continues to increase until around 60 days (Taylor, 1994 chapter 12), hence the wing lengths recorded for offspring in the nest are likely to be smaller than adult size, and the variance of these data is expected to be greater. The consequences of sampling birds of different ages are discussed in chapters 6 and 7, as it introduces possible errors and biases in estimates of the heritability of metric traits, and also introduces problems of interpretation of isozyme patterns. It can be seen from table 3.1 that the young barn owls and a number of adult birds are classed as unknown sex, yet it would be beneficial to know the sex of these birds for both morphometric and isozyme analyses. Possible methods for identifying the sex of barn owls are therefore discussed in the following chapter.

SUMMARY

Two wild and three captive barn owl populations were sampled, with the aim of taking a minimum of 10 samples per population; one wild population was sampled more thoroughly to provide data on related individuals.

'Sampling' involved taking a blood sample for isozyme analysis, measurements for morphometric comparisons, and photographs of plumage for sex determination; feather samples were also taken from some young birds for karyological sex determination.

Wild birds were caught during the breeding season using a hand held net or a box trap; breeding females could be caught relatively easily with the net during incubation, but sampling some males required the more time consuming use of the trap. Offspring were sampled at their natal site before they fledged.

Chapter 4

DISCRIMINATING BETWEEN THE SEXES

4.1 Introduction

The sampling program described in chapter three resulted in blood samples, measurements of body size and photographs of plumage being obtained from a sample of barn owls from two wild and three captive populations. All of the juvenile birds, and a small proportion of the adults, were classed as unknown sex in table 3.1, yet to allow meaningful interpretation of the variation in isozyme frequencies or quantitative traits within and among populations, the analyses should be carried out on individuals of known sex. The sexes could differ in their morphology, behaviour and ecology, and so failing to distinguish between the sexes could result in inflated estimates of within population variation for a particular trait, and possible evolutionary or ecological explanations for the variation would remain unrecognised. In addition, unequal sex ratios in different populations would invalidate direct comparisons of variation among populations. The ability to identify the sex of barn owls in this study would be particularly useful, as sexual dimorphism has been reported for both body size (Marti, 1990) and plumage (Taylor, 1989). To avoid problems of circularity, the ideal method for discriminating between the sexes would be independent of body size variables.

This chapter discusses some possible methods that may be used to discriminate between the sexes in barn owls (4.2), and in more detail describes the extent of sexual dimorphism in plumage (4.3), including the accuracy by which the sex of barn owls may be determined by plumage characteristics.

4.2 Methods for discriminating between the sexes

Unlike the majority of mammals, most birds do not exhibit external differences in their genitalia, and so there is no simple and universal method for identifying the sex of individuals. Available methods reflect sexual differences in body dimensions, reproductive organs, DNA, behaviour, hormones and chromosomes.

The techniques differ in their expense, invasiveness, ease of execution and accuracy; of the characters used, some are unambiguously dimorphic whereas others show some degree of overlap between the sexes; some reflect permanent differences, whereas others may vary with age and season. The ideal method for distinguishing between the sexes in this study would be unambiguous, applicable to birds of any age and in any season, and would be independent of the variables under study. It would involve non-harmful procedures, and would preferably not

involve expensive and time consuming laboratory work. These were the criteria used to assess the suitability of the possible methods described in the following sections.

Body dimensions

Sexual size dimorphism has been used to separate the sexes in a number of avian species (Bortolotti, 1984), and is described in a number of owl species (32 forms of north American owls, Earhart and Johnson, 1970; great horned owl *Bubo virginiatus virginiatus*, McGillivray, 1985; Tengmalm's owls *Aegolius funereus*, Hakkarainen and Korpimaki, 1991). In barn owls, sexual size dimorphism is reported for the traits weight, body length, and tarsus length in adult *T. a. pratincola* in Utah (Marti, 1990), and it is sometimes assumed that a similar dimorphism occurs in the British *T. a. alba* population. Johnson (1991) for instance, explains an apparent sex difference in talon flange width in barn owls over seven months after fledging as 'due to reversed size dimorphism in the species', yet there is evidence that British barn owls are not sexually dimorphic to the extent described for the north American sub-species. Taylor (1994) for instance, found no sexual size dimorphism in measures of live adult birds in his study other than for body weight, the difference being most extreme during breeding when female weight is higher than males due to fat deposits, growth of the reproductive tract and egg weight. (see also chapter 5). Johnson claimed that talon flange width averaged 1.5mm in males and 2.0mm in females seven months after fledging, but without providing the range and variance for these data the significance of this apparent difference can not be assessed. Flange width was in any case reported as not dimorphic in newly fledged birds, suggesting it would have limited value for assigning a sex to barn owls in this study. Morphometric traits were rejected for distinguishing between the sexes in this study of barn owls because a method applicable to both adults and juveniles was required, and because their use would introduce problems of circularity in the interpretation of patterns of variation.

Reproductive organs

Methods which involve the direct assessment of the birds reproductive organs have the advantage that they are 100% accurate if practised by an experienced worker. One method involves inducing the cloaca to prolapse; in the female, the opening to the oviduct is revealed, whereas the male is induced to ejaculate semen, but this is only effective in adult birds. Other methods of cloacal examination are also possible, but all require the birds to be adults in breeding condition. A surgical method (laparotomy) may be employed to examine the gonads of birds of any age, but requires the bird to be anaesthetised (Cooper, 1978); the invasiveness of these techniques limits their use in field studies.

DNA

Recent developments in DNA analysis have provided another method by which the sex of birds may be identified; Griffiths and Holland (1990), for instance, describe sequences of DNA unique to the female W chromosome in lesser black backed gulls (*Larus fuscus*). Unfortunately, the sex specific DNA sequence is thought to be genus specific, and the development of a probe for a different genus would require a considerable investment of time and resources.

Surgical methods and DNA analyses were rejected as unsuitable for this study, but methods based on differences in behaviour, hormones and chromosomes were selected for trial, and are described in detail in the sections 4.2:1- 4.2:3.

4.2:1 Sexual differences in behaviour

During the breeding season, mated pairs of barn owls exhibit behavioural differences due to their different roles whilst rearing young (Taylor, 1994 chapter 12). The female alone incubates, and is readily identifiable by the presence of a conspicuous brood patch, where the belly feathers are lost to reveal an area of bare skin; males do not develop a brood patch (Taylor, 1994, 11.3). The male tends to be the more active of the pair, bringing in food for the female and young chicks. Although both parents commonly roost together during the early breeding stages of pairing and laying, the male is conspicuously more flighty, and either through superior vigilance or lower site loyalty is likely to be the first one caught when a site is approached with the intent to catch the adults (see chapter 3). Some females incubating eggs or brooding small chicks may 'sit tight' and be approached and lifted off their young without any escape response. As the breeding season progresses, males are more likely to be found roosting away from the brood, depending on the availability of suitable roosting sites; the female may also roost away from the brood after the youngest is 12-16 days old. The sex of breeding adults can therefore be accurately determined, the most reliable method being to catch a pair together during laying or incubation, and to identify the female by the presence of her brood patch. This method is of no use for determining the sex of juveniles or non breeding adults, however, and so alternative methods were investigated.

4.2:2 Steroid hormones

(a) Chromatographic separation

Dieter (1973) reported the successful identification of sex in eagles, owls and herons by the chromatographic separation of steroid hormones from blood samples, a method which appeared attractive initially as it was described as suitable for any age and irrespective of breeding condition, and had the additional advantage that blood plasma surplus to the

requirements for isozyme analysis could be used. The steroid hormones are separated by centrifugation through a silica gel column, and are detected as dark bands when the column is incubated with iodine.

Trials using blood samples from chickens, turkeys and barn owls were therefore undertaken; details of the methodology and results are presented in appendix 2. This method proved unsatisfactory for determining sex in the trial samples, and was not therefore pursued further.

(b) Radioimmunoassay (RIA)

Despite the apparent lack of sensitivity in the above method, the idea that both immature and mature birds could be sexed due to differences in steroid levels led to the use of a more sensitive technique for assaying hormone levels. Bercovitz and Sarver (1988) demonstrated sex related differences in sex steroids from day old Andean condors *Vultur gryphus* and peregrine falcons *Falco peregrinus* by radioimmunoassay from allantoic egg waste samples, indicating that this may be a suitable method for both adults and very young birds.

The technique involves the competition for a limited number of antibody binding sites between a finite amount of radio-labelled antigen and the unlabelled antigen (e.g. a steroid hormone) of the test sample. The greater the concentration of antigen in the sample under test, the less radio-labelled antigen is bound to the antibody; the bound and unbound antigen may be separated by chromatography, and the radioactivity of the free antigen measured. The concentration of the hormone in the test sample can then be determined by reference to a calibration curve made with known concentrations of antigen (Burrin, 1986).

A pilot study to test the applicability of using RIA from plasma samples to distinguish between the sexes in barn owls was carried out; this technique can detect far lower concentrations of testosterone and β-estradiol than the chromatographic method described above. β-estradiol was assayed by the Reproductive Endocrinology Laboratory, Centre for Reproductive Biology, Edinburgh University; testosterone was assayed by the Immunoassay section, Department of Clinical Chemistry, Edinburgh University. Approximately 50µl plasma is required for this assay.

The results are presented in table 4.1. The β-estradiol assays revealed concentrations of less than 100 pmol/l for all males, non breeding females, and juveniles of either sex. The two breeding females had higher concentrations, of 166 and 352 pmol/l. Low testosterone concentrations of 300 pmol/l or less were found in non breeding adults and juveniles; breeding

birds of both sexes had higher concentrations, with one wild male, recuperating from a recent road injury in captivity, having the highest level of 3700 pmol/l.

Table 4.1 Results of Plasma Hormone Assays: Concentrations of two steroid hormones, β-estradiol and testosterone, determined by radioimmunoassay from barn owl blood samples.

Sample	β-estradiol pmol/l	Testosterone pmol/l
wild adult male, breeding	<100	600
wild adult female, breeding	352	1300
wild adult female, breeding	166	700
wild/captive adult male, non breeding?	<100	3700
captive adult male, non breeding	<100	-
captive adult male, non breeding	<100	-
captive adult male, non breeding	<100	-
captive adult male, non breeding	<100	-
captive adult male, non breeding	<100	-
captive adult male, non breeding	<100	-
captive adult male, non breeding	<100	-
captive adult male, non breeding	<100	-
captive adult female, non breeding	<100	-
captive adult female, non breeding	<100	-
captive adult female, non breeding	<100	-
captive adult female, non breeding	<100	-
captive adult female, non breeding	<100	-
captive adult female, non breeding	<100	-
captive adult female, non breeding	<100	-
captive adult female, non breeding	<100	-
wild adult, sex unknown, non breeding	<100	<300
wild adult, sex unknown, non breeding	<100	<300
wild adult, sex unknown, non breeding	<100	<300
wild adult, sex unknown, non breeding	<100	<300
Juvenile (Female?- by plumage)	100	<300
Juvenile (Female?- by plumage)	<100	<300
Juvenile (Male?- by plumage)	<100	<300
Juvenile (Female?- by plumage)	<100	300

The results imply that relatively high β-estradiol levels (~166+ pmol/l) may allow the identification of sex in adult female barn owls during the breeding season, but as the hormone is at far lower concentrations in non breeding adult females and in juveniles of 50-60 days old (<100 pmol/l), these birds are indistinguishable from males. A seasonal production of β-estradiol in adult female barn owls is suggested; similar variation in the production of steroid

hormones has been documented in other avian species (Lincoln et al., 1980), together with considerable individual variation in hormone levels.

Testosterone levels would not appear useful for distinguishing between the sexes from these results; although the highest level was in a wild male (held in captivity due to a broken wing- breeding status therefore unknown), the two wild breeding females had higher testosterone concentrations than the one wild breeding male assayed.

Hormonal differences in very young birds may be a function of the embryonic mechanism of sexual differentiation (Bercovitz and Sarver, 1988). The lack of differentiation among the juvenile barn owls in this assay may therefore be due to their age; steroid hormones may be useful for identifying the sex of neonatal young or breeding adults, but intermediate aged immatures may not exhibit detectable differences in their steroid hormone levels.

In summary, this method appears suitable for identifying the sex of breeding female barn owls. As they are in any case easily recognisable by the presence of a brood patch (see 4.2:1), this method was rejected.

4.2:3 Chromosomes

Karyotyping provides another potential method for identifying the sexes. In barn owls the female W chromosome is small and acrocentric; the Z chromosome is not only the largest of all the chromosomes (2n = 92), but the only one that is metacentric (Belterman and De Boer, 1984). The sex of an individual could therefore be accurately determined by the number of Z chromosomes on a karyogram. Chromosome preparations may be made by the culture of lymphocytes from peripheral blood; Belterman and De Boer (1984) describe successful cultures using avian blood up to four days after removal, if the blood is stored at 4° C.

Preliminary trials of cell culture on barn owl plasma samples, carried out at the Western General Hospital, Edinburgh, indicated that cell culture was not reliable on samples more than a few hours old. This made the technique impractical for use on samples collected in the field, due to the travel times involved between study sites.

Chromosome preparations may be made from sources other than blood samples, however, and Databird Worldwide Ltd. (Denton, Peterborough PE7 3SD) operate a karyological bird sexing service which involves the preparation of cell cultures from blood feathers. Growing feathers, at the 'pin' stage when only a small amount of feather is unfurled from the quill, have actively dividing cells at their base which can be cultured. The feather is carefully pulled, and stored,

refrigerated, in collecting tubes supplied by Databird; successful culture is likely if samples of a suitable quality are despatched by courier to arrive at their laboratory within seven days from sampling. As growing feathers are required, this is most useful for young birds growing their first feathers; flight or tail feathers are recommended. For adults not undergoing moult, a grown feather must first be pulled and discarded, and its replacement pulled during growth. This was not feasible for the wild adults in this study, but a trial of ~30 samples was undertaken for young pulli.

Sampling the recommended flight or tail feathers at a young enough age to allow complete regrowth before fledging would have involved additional site visits when the young were still being brooded by the female. To sample all the young at the required age would have increased site visits to a frequency exceeding that determined by Taylor (1991) to be unharmful to the birds, and would have considerably increased the time and cost of sampling. To minimise site visits, therefore,, samples of pin feathers from the coverts were sampled when the birds were of a suitable age to be ringed, approximately 35 days old, taking larger feathers in preference (~4cm long). These proved not to be suitable for cell culture, however, and the problem of finding an independent method for identifying the sex of non breeding barn owls remained unsolved.

There remained the possibility, however, that some of the unknown sexed birds could be assigned a sex on the basis of their plumage; the use of plumage to distinguish between the sexes is discussed in section 4.3.

4.3 Sexual dimorphism in plumage

It was apparent from catching and handling the breeding pairs of wild barn owls that plumage differences could allow the accurate separation of the sexes in some cases; indeed a subjective assessment of plumage is usually the basis for identifying the sex of captive birds for breeding purposes. My aim was to quantify aspects of plumage variation, to identify components of the plumage which best discriminated between the sexes and to assess the accuracy of possible plumage methods by testing their predictive abilities on known sex individuals from different populations.

In general, female barn owls of the *T. a. alba* subspecies tend to be darker in colour overall, the upperparts being a darker buff and with more pronounced markings on the wing and tail feathers than the males. Whereas the males are usually white on their underparts, females may have traces of buff colouring; flecking of the females' underparts is also usually more heavy

than in males, with the flecks larger, more numerous, and extending over a larger area of the plumage.

The problem here is that although generalisations may be made concerning typical males and females, some overlap in characteristics occurs, and so a proportion of the birds are likely to be misclassified.
This may be partly attributable to age effects, as a bird's colouration and markings may change with age; newly fledged barn owls have more extensive buff colouration on their underparts, and more flecks, than adult birds. Taylor (1994, 2.1) reports that 44% of newly fledged males in his study area had faint buff on their chests, but only 12% of these birds had retained the colouring when captured the following year. Taylor notes that females continue to become paler as they get older, such that females several years old could be mistaken for males.

Taylor (1994, 2.1) found the most reliable character for identifying the sex of adult birds was the flecking of the underparts, and devised an index to categorise the extent of flecking as follows:

0= Unflecked
1= Small number (<10) of small flecks on flanks only
2= Dense flecking on flanks
3= Flecks extending from flanks to side of chest
4= Flecks covering flanks, side and centre of chest

Accuracy of Taylor's index from photographs and in the field
Taylor assigned a plumage index score to barn owls at the time of capture. 98% of females (n=182) in Taylor's study had some flecking on their underparts (scores 1-4), whereas 95% of males (n=149) had no underbody markings (score 0), and 98% had no underwing flecks. Female flecking was extensive in the majority of cases, with large flecks occurring over much of the underparts (scores 3 and 4).

The distinction was not so clear for newly fledged birds, however, with fewer birds being recorded as males when the adult criteria were applied. Taylor (1994, 2.1) found that all of the newly fledged birds with a score of 0 that were subsequently caught as breeding adults proved to be males, and those with scores of 3 or 4 proved to be females. The method could therefore be usefully applied to young birds, but scores of 1 and 2 are ambiguous.

In this study, photographs of underbody and wing plumage were taken at the time of blood sampling (see chapter 3) to allow flexibility in quantifying plumage characters. Working from the slides projected onto a white screen, each known sex barn owl for which a good quality slide was available was assigned an index score. The results are presented in table 4.2.

Table 4.2 Flecking index scores derived from photographs of known sex adult barn owls in populations 1-5

Population	Flecking index score					n
	0	1	2	3	4	
Population 1 males	10	4				14
Population 1 females	5		5	13	4	27
Population 2 males	6	4				10
Population 2 females	4	2	2	3	3	14
Population 3 males	7					7
Population 3 females	2	2	1	1	1	7
Population 4 males						0
Population 4 females				1		1
Population 5 males	2					2
Population 5 females		4				4

In the Langholm population (population 1), which corresponds to Taylor's study area, 71.4% of males (n = 14) had no underbody markings according to the photographs, compared to 95% of males assessed by Taylor in the field. All males had a score of either 0 or 1; males in the photographs had either no flecks or very few flecks. 81.5% of females in the photographed sample (n = 27) had at least some flecking, with most females having the extensive flecking score of 3; this compares to 98% of females in Taylor's study. If the cut off point for sex identification is taken as between a score of 1 and 2, 5 out of the 41 known sex adults in the photographed sample of population 1 are misclassified, giving an overall accuracy of 87.8%.

From this comparison, the flecking index appears more ambiguous when derived from photographs than from birds in the field. One possible explanation for this discrepancy would be that that flecking is less accurately assessed from photographs than from live birds; overexposure of photographs could reduce the apparent flecking, resulting in females being mis-classified. This is unlikely, however, as photographs were assessed for their quality; each frame included a standard reference card, and exposure was judged to be good if the faint blue ruled lines on the card were clearly visible. The few slides where this was not the case were

excluded from plumage analysis. It would also be difficult to explain mis-classified males in terms of photograph quality, as in their case the photographs revealed a greater amount of flecking than would be predicted from the live bird sample. The difference in accuracy may still be partly explained in terms of a careful examination of birds in the hand revealing flecking that could be obscured by overlapping feathers in the photographs, or it may simply be an artifact of sampling, with atypical birds having a greater influence on the predictive ability of the index in the smaller sample of photographed birds.

Population differences in flecking index scores

Taylor's index is potentially very useful for assigning a sex to unknown sex birds in this study, but is only appropriate if it is applicable to populations other than the one in Scotland where it was devised and tested. If the cut off point (between scores 1 and 2) is applied across all the populations in this study (see table 4.2), the accuracy is somewhat lower than in population 1, with 19 of the 86 birds misclassified; in each case, misclassification involves females being wrongly classed as males, giving an overall accuracy of 77.9%.

This reduction in the predictive value of the index when different populations are pooled suggests that the degree of sexual dimorphism in flecking may not be universal among populations. Sample sizes were too small to allow testing among the populations, but a significant difference between the number of females with scores of 0-1 and scores of 2-4 was found between the Langholm population and populations 2-5 combined ($X^2 = 7.03$, df1 ***).

The differences between populations may not be explained by photograph quality, as this was consistent among populations. Presuming no errors in the independent methods of sex determination, it can be concluded that methods of identifying the sex of barn owls by plumage differences should not be extrapolated to other populations without testing their validity; a larger sample size per population would be required to accurately quantify any population differences.

Multivariate analysis of plumage characters

Multivariate techniques which take a number of characters into account may be useful in distinguishing between the sexes when some overlap occurs between the sexes (Schlinger, 1990). This would allow, for instance, the size, shape and distribution of flecks to be taken into consideration. As flecking is just one aspect of the plumage which exhibits some degree of sexual dimorphism, a multivariate analysis which also included other characters could improve the predictive value of the plumage for sex identification. As the degree of sexual dimorphism exhibited by other characters could be more consistent than the flecking index in different populations, a method of sex identification which is more universally applicable could also be devised by multivariate techniques. Plumage variation was therefore investigated in more detail, to include details of coloration as well as the size and shape of markings. Of the 40 available slides of unknown sex barn owls, 21 were from population 1, representing 18 young birds and three non breeding adults. It was therefore of particular interest to devise a method which could accurately identify the sex of birds in population 1; this is discussed below.

Slides of underbody and wing plumage of both adult and juvenile birds were projected onto a white screen, and markings and coloration were assessed for the 14 plumage regions illustrated in figs. 4.1 and 4.2, and also for beak, feet and claws. The regions were chosen to represent naturally defined plumage areas which could be readily identified in photographs of birds held at slightly different angles, and approximate the areas described in Taylor's flecking index.

Each region was assigned a score of increasing value with increasing overall darkness of colour; fig. 4.3 shows the scales used for each region. In all but the two smallest plumage regions of the face, the score represents the two most dominant colours present, hence an area of the wing whose only coloration was moderate buff was given a score of 3, whereas if some light buff was also present, the score was 2, irrespective of the amount of white plumage present. The extent of body or wing colouring was treated separately, with each region assessed for the approximate proportion of the area covered by coloured plumage. The head regions were far smaller than the body and wing plumage areas, and so it was not attempted to assess the proportion of each area occupied by the individual colours. In this case, white plumage was included as a colour, giving a score of '1' if no other coloration was present.

Flecking was quantified as the number of flecks belonging to each size/shape class found in each area. The size of the flecks was assessed visually, with reference to a standard sized

reference card included in each photograph; their defining measurements should therefore be taken only as approximations of true size.

The quality of each photograph was also assessed, according to whether it was over exposed, good, or underexposed, by assessing the clarity of the blue ruled lines of a standard reference card included in each photograph. Few were of poor quality, and these were excluded from subsequent analyses.

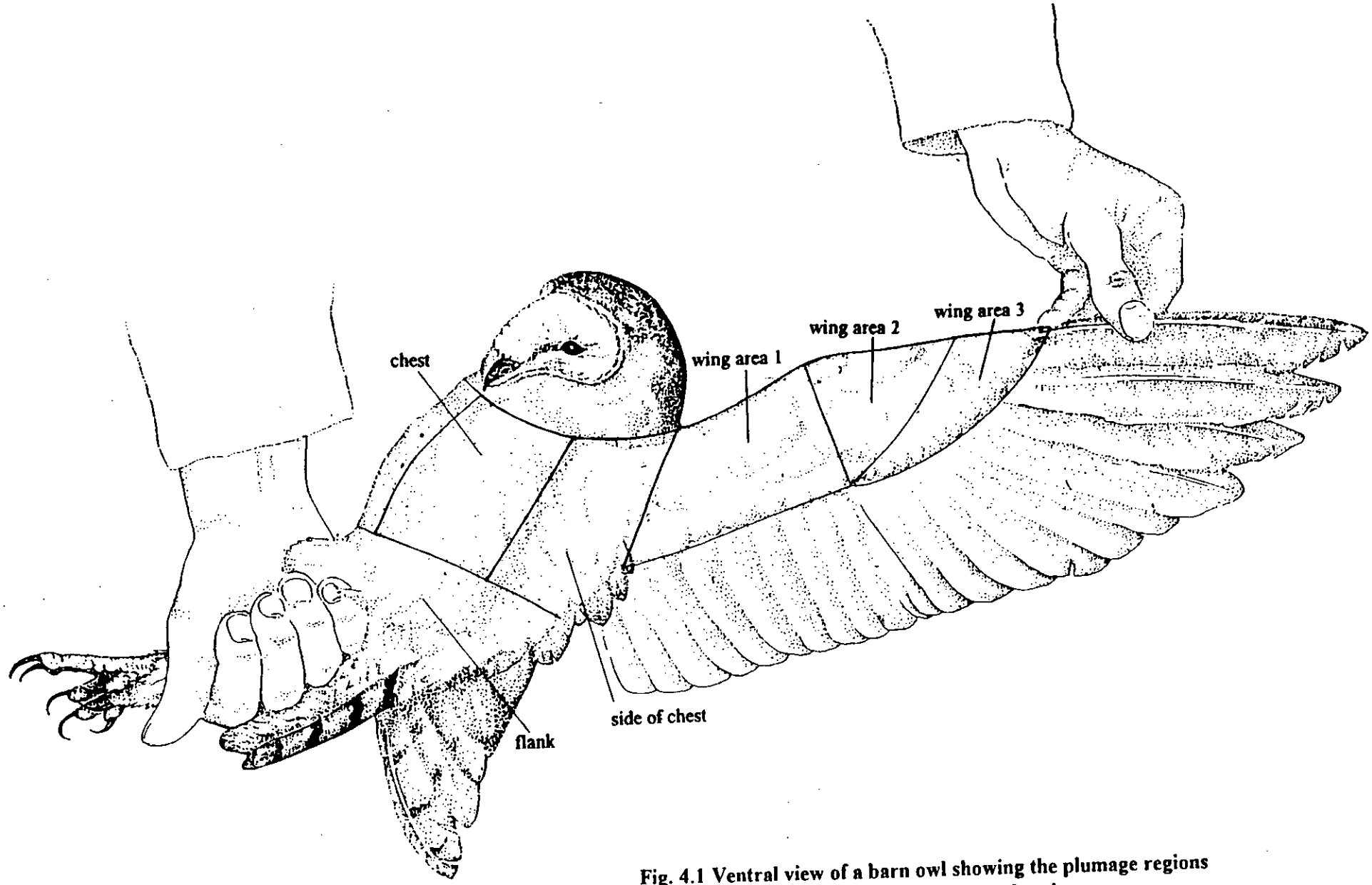


Fig. 4.1 Ventral view of a barn owl showing the plumage regions of the body and underwing

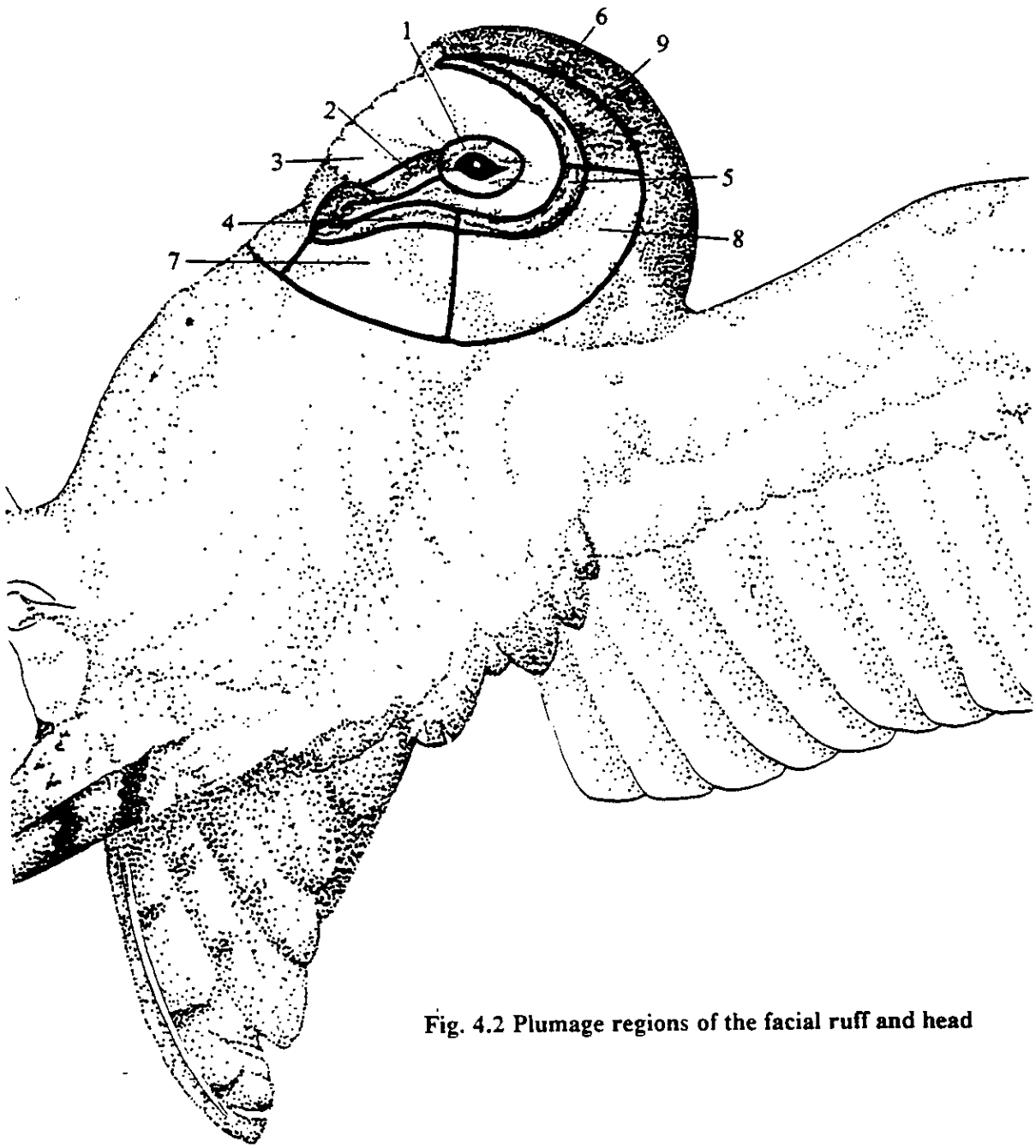


Fig. 4.2 Plumage regions of the facial ruff and head

Fig. 4.3 Scores for plumage characters assessed from ventral view slides of barn owls

PHOTOGRAPH QUALITY 1=Bright, 2=Good, 3=Dark

COLOUR OF HEAD REGIONS 1 AND 2; 0=white all over

	Light buff	Moderate buff	Dark buff/black
Small area (<0.50)	1	3	5
Large area (>0.50)	2	4	6

COLOUR OF HEAD REGIONS 3-9

	White	Light buff	Moderate buff	Dark buff	Grey	Black
White	1	2	4	7	11	16
Light buff		3	5	8	12	17
Moderate buff			6	9	13	18
Dark buff				10	14	19
Grey					15	20
Black						21

BEAK/FEET/CLAW COLOUR

	Ivory/pale yellow	Bright yellow	Grey	Black
Ivory/pale yellow	1	2	4	7
Bright yellow		3	5	8
Grey			6	9
Black				10

EXTENT OF BODY/WING COLOUR

0	No colouring
1	Traces
2	Approx. 0.25 of area
3	Approx. 0.50 of area
4	Approx. 0.75 of area
5	Entire area

BODY/WING COLOUR 0=white all over

	Light buff	Moderate buff	Dark buff	Grey
Light buff	1	2	4	7
Moderate buff		3	5	8
Dark buff			6	9
Grey				10

FLECKS

Record the number in each of the six body and wing areas:

- Small (<1mm in length, where 'length' is determined as the distance across the mark, parallel to the feather rachis)
- Medium streaks (1-2mm in length, very narrow in width in comparison to length)
- Large streaks (2+mm in length)
- Medium spots (1-2mm in length, width similar to length)
- Large spots (2+mm in length)

Data were collected for 54 characters; they were the colouration of nine head regions and beak, feet and claw colour; the colouration, and the extent of colouration of six body and wing regions and the number of each of five classes of fleck in each of the six body and wing regions. As few birds had markings classed as 'streaks', the two size classes for this character were combined, resulting in 48 characters in total. These are listed in table 4.3.

The number of flecks present for both sexes, summed over areas and size classes, is summarised in the histograms in fig. 4.4 and 4.5. As would be expected from the index data, although males generally have fewer flecks than females, there is no obvious cut off point between the sexes for this character, as some females also have low fleck counts. All but one male had less than 30 flecks overall, and more heavy flecking is characteristically female. As this simple combination of variables did not aid in the identification of the sex of intermediately flecked birds, all data were submitted to a multivariate analysis.

To find the sub-set of variables which best characterised the differences between the sexes, the data were analysed by stepwise discriminant function (DF) analysis, using BMDP7M Statistical software (1990). The P7M program enters one variable at a time into the classification function, until the group separation ceases to notably improve. A univariate analysis of variance is initially made for each variable, and the variable for which the means differ the most is entered as the first discriminating variable. In the following steps, the variables which have already been included in the function are taken into account in determining the next variable to be entered; the process is continued until little improvement in classification is gained by adding more variables (Dixon, 1983). As the model assumes normal distributions for all variables, data were log transformed where appropriate. The program computes the Mahlanobis D^2 for each case, which describes the distance of each case (bird) to the centre of each group (sex), and the posterior probability of the case assigned to each group, which gives an indication of how likely it is for each bird to be male or female, based on the plumage characters used. On the basis of this, each bird is assigned a sex.

The sex of birds could be entered as male, female or unknown, hence the ability of the model to distinguish between the sexes could be tested on known sex birds, and the predictive power of a classification based on one population for other populations could be tested by setting the known sex birds of the other populations as unknowns. In each trial, a 'jacknife classification' was used; for each case, the classification function is computed with the data omitted for the bird under test, hence the function is not classifying the same cases that were used to compute it. This gives an indication of the model's predictive power to assign a sex to unknown individuals, if they do not deviate in character from the defining population.

Table 4.3 Colour and marking characters used in analysis of barn owl plumage

Character	Description
H1	colour and extent of colour in head region 1 (0-6)
H2	colour and extent of colour in head region 2 (0-6)
H3	one or two predominant colours in head region 3 (1-21)
H4	one or two predominant colours in head region 4 (1-21)
H5	one or two predominant colours in head region 5 (1-21)
H6	one or two predominant colours in head region 6 (1-21)
H7	one or two predominant colours in head region 7 (1-21)
H8	one or two predominant colours in head region 8 (1-21)
H9	one or two predominant colours in head region 9 (1-21)
B	colour of beak (1-10)
F	colour of skin on feet (1-10)
C	colour of claws (1-10)
ES	extent of colouring in the flank region (0-5)
EC	extent of colouring in the breast region (0-5)
EF	extent of colouring in the leg region (0-5)
E1	extent of colouring in wing area 1 (0-5)
E2	extent of colouring in wing area 2 (0-5)
E3	extent of colouring in wing area 3 (0-5)
CS	one or two predominant colours in the side of chest region (1-21)
CC	one or two predominant colours in the chest region (1-21)
CF	one or two predominant colours in the flank region (1-21)
C1	one or two predominant colours in wing area 1 (1-21)
C2	one or two predominant colours in wing area 2 (1-21)
C3	one or two predominant colours in wing area 3 (1-21)
SS	number of small (<1mm) flecks in the side of chest region
SL	number of streaks (1mm+) in the side of chest region
SMS	number of medium (1-2mm) spots in the side of chest region
SLS	number of large (2mm+) spots in the side of chest region
CS	number of small (<1mm) flecks in the chest region
CL	number of streaks (1mm+) in the chest region
CMS	number of medium (1-2mm) spots in the chest region
CLS	number of large (2mm+) spots in the chest region
FS	number of small (<1mm) flecks in the flank region
FL	number of streaks (1mm+) in the flank region
FMS	number of medium (1-2mm) spots in the flank region
FLS	number of large (2mm+) spots in the flank region
1S	number of small (<1mm) flecks in wing area 1
1L	number of streaks (1mm+) in wing area 1
1MS	number of medium (1-2mm) spots in wing area 1
1LS	number of large (2mm+) spots in wing area 1
2S	number of small (<1mm) flecks in wing area 2
2L	number of streaks (1mm+) in wing area 2
2MS	number of medium (1-2mm) spots in wing area 2
2LS	number of large (2mm+) spots in wing area 2
3S	number of small (<1mm) flecks in wing area 3
3L	number of streaks (1mm+) in wing area 3
3MS	number of medium (1-2mm) spots in wing area 3
3LS	number of large (2mm+) spots in wing area 3

Fig. 4.4: Total number of flecks recorded from photographs of underparts, for adult male barn owls. The birds were sexed by breeding records or behaviour, as described in section 4.2:1; data from all five study populations are combined.
n = 33; bar width = 10 flecks.

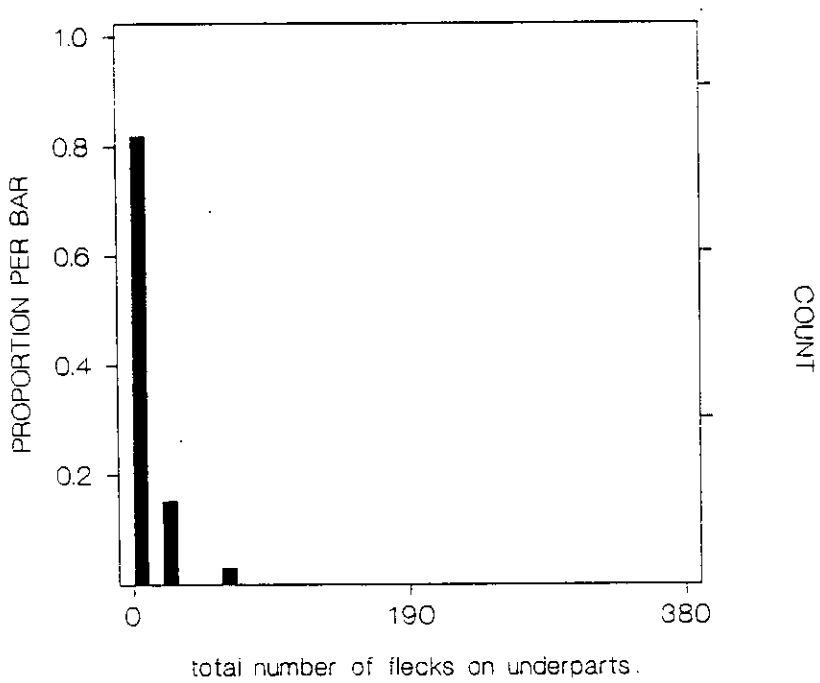
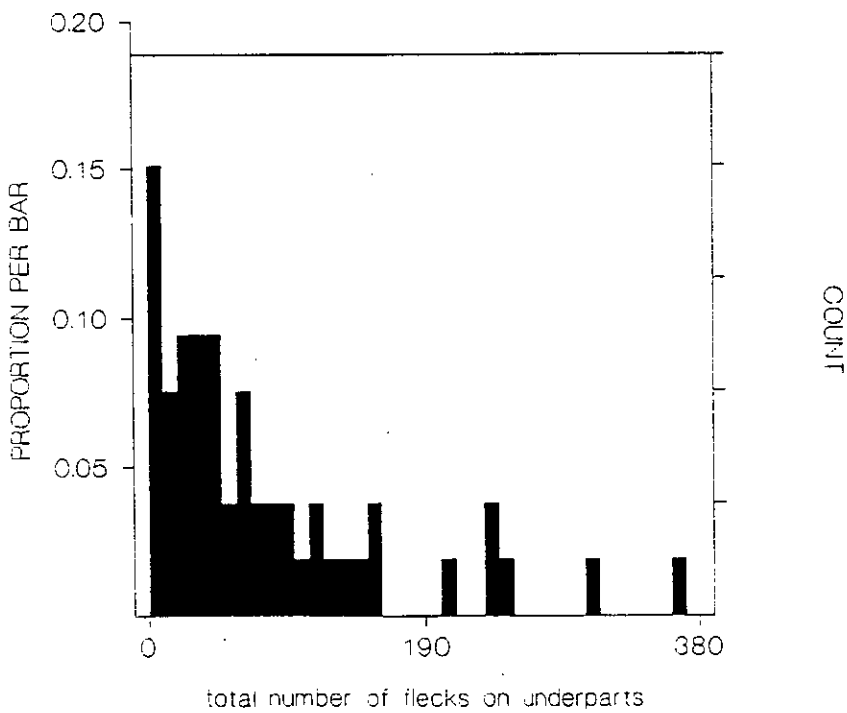


Fig. 4.5: Total number of flecks recorded from photographs of underparts, for adult female barn owls. The birds were sexed by breeding records or behaviour, as described in section 4.2:1; data from all five study populations are combined.
n = 53; bar width = 10 flecks.



(a) All known sex adults used to compute the classification function

Five variables were used in this classification: 2S, H9, FS, C and EF, i.e. the characters which best described sexual dimorphism involved the number of small spots in wing area 2, colouring in head area 2, the extent of colouring and the number of flecks in the flanks, and claw colour. The jacknifed classification predicted 12 of the 86 known sex adults incorrectly; 3 males were classed as females, 9 females were classed as males, giving 90.9% males correct, 83.0% females correct, and 86.5% of barn owls correctly sexed overall.

Of the incorrectly classified birds, three females and one male were from population 1, giving 92.9% males correct, 88.9% females correct, and 90.2% of birds from population 1 classified correctly overall. Of the remainder, five misclassified birds were from population 2 (n=24), one from population 3 (n=14) and two from population 5 (n=6).

Of the nine incorrectly sexed females, four were first year birds, and the oldest of known age was a captive bird of four years old. Two birds were 2 and 3 years old, and two were of unknown age. Although the sample size is small, the age structure does not indicate that it is predominantly older females that are misclassified as males in this sample, as would occur if their plumage had become paler as they aged.

The Mahlanobis D^2 posterior probabilities for the three mis-classified males being females in this analysis were 0.586, 0.752 and 0.991; only one showed a probability close to the borderline of 0.500, whereas the other two known sex males showed a high probability of being female on the basis of their plumage.

The Mahlanobis D^2 posterior probabilities for the nine mis-classified females were: 0.537, 0.603, 0.607, 0.608, 0.653, 0.803, 0.915, 0.935, 0.959. As with the mis-classified males above, not all the mis-classified birds had plumage scores which would make their designation to a sex ambiguous; some have a very high probability on the basis of their plumage of being the opposite sex to what is known from their breeding record. This suggests that a proportion of the sampled barn owls had strongly atypical plumage for their sex; not all incorrectly sexed birds would have intermediate plumage characteristics.

(b) Known sex adults from population 1 used to compute the classification function

The analysis was repeated, classifying all known sex adults from populations 2-5 as unknown birds, hence the classification function was based on the characteristics of known sex adults in population 1 only.

The characters selected this time were H1, H8 and Log1S, i.e. characters involving the colouration of the facial disc and side of the head, and the number of small flecks in wing area 1. Two males and three females were misclassified in population 1, giving 85.7% males correct, 88.9% females correct, and 87.8% correct in population 1 overall.

When this classification was used to test the known sex birds of the other populations, little difference in the merits of this classification and that based on all known sex barn owls was found. In this case, four misclassified birds were from population 2 (n=24), four were from population 3 (n=14) and one was from population 5 (n=6). This gives a total of 14 birds misclassified from all populations, an accuracy of 83.7%, compared to 12 misclassified (86.5%) when the discriminant analysis is based on all known sex barn owls.

Comparing the plumage methods for discriminating between the sexes

The accuracy of the plumage methods described in this chapter is summarised in table 4.5. Taylor's index, when applied by Taylor in the field gave a higher success in predicting the sex of known sex individuals in his study of 331 barn owls, than any of the methods applied to the sample of barn owls in this study. The same index applied to photographed samples, however, indicated that this index, although highly accurate for the population for which it was devised, is less accurate when other populations are sampled; in addition, Taylor (1994) noted that the flecking index may not be strictly applicable to young birds, as there is a tendency to overestimate the number of females in the population in this case. No such bias was found with the discriminant function analysis, despite the inclusion of a flecking variable; possibly the other characters included do not vary as much with age. The discriminant function analysis also appears more suitable for use in other populations than the flecking index, possibly because the characters used exhibit fewer population differences. A larger sample of young and adult birds that can be sexed by some other means would be required to clarify these points.

The predictive abilities of Taylor's index and discriminant function analysis did not greatly differ when used on plumage data collected from slides. A bias towards wrongly classifying females as males may be due to the use of photographs, resulting in an underestimation of flecking on some females. When Taylor's index is applied, as it was intended, to live birds in the field, the accuracy of the technique may be greater than achieved by the discriminant function analyses used here, but could possibly be improved by the inclusion of additional characters, such as facial disc and side of head colouration.

Table 4.5 The percentage of known sex barn owls correctly identified by four analyses of plumage characteristics

DF = discriminant function analysis

Method	Birds in population 1		All birds	
	n	% correct	n	% correct
Taylor's index in field (Taylor, 1994)	331	96.7%	-	
Taylor's index from photographs (this study)	41	87.8%	86	77.9%
DF (based on all birds)	41	90.2%	86	86.5%
DF (based on popn. 1 only)	41	87.8%	86	83.7%

Designating a sex for unknown sex individuals

Birds sexed by Taylor in the field on the basis of their plumage index score were included in this study. On the basis of the above analyses, the 40 barn owls of unknown sex for which photographs were available were assigned a sex using a discriminant function analysis based on known sex birds from all the study populations. Of a total of 24 juvenile birds, 12 were classified as males, and 12 as females in the discriminant function analysis. Although the validity of using a classification based on adult birds for the juveniles can not be tested without an independent measure of sex for the young birds, this sex ratio is at least an indication that this method is appropriate. Further evidence is provided by a single juvenile which was subsequently caught as a breeding adult, and was correctly classified as a male.

Table 4.6 shows the number of birds assigned a sex in this study. A total of 269 barn owls were assigned a sex by one of the methods described in this chapter. Of these, breeding data accounted for 180, and so 89 extra birds were assigned a sex on the basis of their plumage. Taylor supplied the sex of 49 individuals for which photographs were not available. Of the birds assigned a sex on the basis of their plumage, 72 were juvenile birds. Only four wild individuals which were sampled as juveniles were subsequently caught as breeding adults (all had been sexed correctly at the time of sampling), yet the sex of juveniles was required for heritability analysis (see chapter 6). The plumage methods described here therefore allowed this analysis to proceed.

Table 4.6 The number of birds assigned a sex in this study by various methods. BREEDING denotes wild birds sexed by the presence or absence of a brood patch, and by behavioural differences as described in section 4.2:1; captive birds are known breeders, including some juveniles for whom breeding data were subsequently available. DF denotes the discriminant function analysis of plumage characters as described in section 4.3. TAYLOR denotes the birds assigned a sex by I.R.Taylor in the field. A = adult, J = juvenile, M = male, F = female, T = total, P = population

P	BREEDING				DF				TAYLOR				TOTAL			
	A		J		A		J		A		J		A		J	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
1	26	44	0	0	1	2	9	9	1	0	20	28	28	46	29	37
2	21	34	0	0	1	1	1	1	0	0	0	0	22	35	1	1
3	11	13	1	1	3	1	2	0	0	0	0	0	14	14	3	1
4	4	10	3	2	2	5	0	0	0	0	0	0	6	15	3	2
5	4	4	1	1	0	0	0	2	0	0	0	0	4	4	1	3
T	66	105	5	4	7	9	12	12	1	0	20	28	74	114	37	44

SUMMARY

Almost all of the juvenile barn owls, and some of the adults sampled from the five populations described in chapter 3 were of unknown sex, yet differences between the sexes may affect analyses of within and among population variation in isozyme frequencies or quantitative traits.

Alternative methods for discriminating between the sexes were therefore considered; surgical techniques and DNA analyses were rejected as impractical for the purposes of this study, but sexual differences in behaviour, steroid hormones, karyotype and plumage were further investigated. Behavioural differences allowed the sex of breeding adults to be identified, as did the radioimmunoassay of estradiol. Karyotyping, which should be applicable to birds of any age, proved unsatisfactory due to a failure in the culture of cells derived from either blood or growing feathers.

The merits of a simple index of underbody plumage flecking, and a multivariate analysis of underbody characters including colouration and the size and number of flecks in different plumage regions, were compared by testing their predictive abilities on individuals sexed by behaviour or breeding records. Taylor recorded an accuracy of 96.7% for his index when applied to a sample of 331 barn owls in the field, but the accuracy of this index, when applied by me to photographs of barn owls was lower; 87.8% of birds (n = 41) were correctly identified. Little difference was found between the index and discriminant function analysis when tested on birds in population 1, with 90.2% correct based on a discriminant function analysis. When applied across all 5 populations, however, the discriminant function analysis had a higher accuracy of 86.5% compared to 77.9% with the flecking index, suggesting population variation in the extent of underbody flecking which could be offset by the inclusion of other plumage characters in the discriminant function analysis. Using the methods described in this chapter, a total of 269 barn owls were assigned a sex; 180 by breeding data and 89 on the basis of their plumage; 40 of those assigned a sex on the basis of their plumage were by the discriminant function analysis and 49 by Taylor.

Chapter 5

VARIATION IN QUANTITATIVE TRAITS

5.1: Introduction

Components of body size are measured on a continuously varying scale. Such traits are termed quantitative, as opposed to the discrete categorisation of qualitative traits. Behavioural and physiological traits may also be quantitative; even traits such as clutch size may be treated as if they were continuously varying, if it is accepted that they represent threshold expressions of an underlying continuum.

Quantitative data may be easy to obtain in a population study, yet the interpretation of such data is not straightforward. The continuous variation in quantitative traits is brought about by a combination of the simultaneous segregation of many genes affecting the character, and non-genetic factors blurring the effects of segregation. The observed phenotypic variance in a population may therefore be thought of as the sum of the genotypic and environmental variance:

$$V_P = V_G + V_E$$

(Falconer, 1981)

Both the genetic and environmental components of variance are likely to differ among traits; even where traits may be expected to covary due to shared genes, such as with components of body size, environmental sources of variation may obscure any genetic relationship among the traits. Tarsus length in barn owls, for instance, is a skeletal trait which remains constant once adult size has been attained when the pulli are approximately 40 days old (Taylor, 1994); any environmental component to tarsus length therefore reflects conditions during skeletal development. Wing length, in contrast, consists of a skeletal component plus feather length; feather extension of the developing pulli continues after adult skeletal size is attained, hence barn owls may not complete full wing length until after fledging. In addition to developmental sources of variation, wing length may vary in adult birds due to primary moult and feather abrasion. Weight has still more sources of variation; it is a compound measure including the weight of skeleton, organs, nutrient stores and reserves, water content and ingested food, and may therefore be expected to vary diurnally and seasonally. Partitioning quantitative variation into its constituent genetic and environmental components has long been the aim of animal breeders and evolutionary biologists; the proportion of the variability in a trait which is due to the additive effects of genes is termed the heritability (see chapter 6).

A study of quantitative traits is potentially a powerful conservation management tool, as most of the characters of evolutionary interest in birds are of a quantitative nature. In this chapter, three approaches to analysing patterns of quantitative variation are taken, as examples of how such data are of interest in a conservation management context.

The first approach is to assess the suitability of the traits measured in this study for heritability estimates. The heritability of quantitative traits is of interest if the aim is to monitor possible loss of genetic variability in small populations, or predict the populations ability to respond to selection (Lande and Barrowclough, 1987) by quantifying variability in traits which are directly related to fitness and survival in the field. Interpretation of the observed patterns of phenotypic variation depends on partitioning the variance into genetic and environmental components; calculating heritability using data from related individuals is the common approach to this problem.

5.2: To assess the suitability of the traits studied for heritability analysis, correlations among the traits are first investigated in section 5.2:1. A strong correlation among the traits would suggest that they are covarying components of a single trait (e.g. body size), in which case no benefit would be gained by monitoring all the traits, and so the handling time of the barn owls in subsequent studies could be reduced by taking a single measurement. In 5.2:2, the measurement error is calculated for each trait; measurement error may be expected to differ among traits due to differences in the instruments used and the ease with which the bird is manipulated for each one. Traits with low measurement error are preferred for heritability estimates, as measurement error sets the upper limit to heritability estimates (Falconer, 1981). In 5.2:3, the phenotypic variation for each trait is described in terms of the phenotypic coefficient of variation, to establish which traits show the greatest levels of variation; traits with very low coefficients of variation may be considered unsuitable for heritability analysis (Boag and van Noordwijk, 1987). Patterns of variation in each trait with respect to sex, age and population are then investigated, as differences among these categories would have implications for the way heritability is estimated (see chapter 6). The implications of the analyses in this section for subsequent heritability estimates are summarised in 5.2:4.

5.3: The second approach to analysing patterns of quantitative variation is to measure departures from bilateral symmetry, as a method of assessing phenotypic quality (Polak and Trivers, 1994). A departure from symmetry has been attributed to inbreeding (Polak and Trivers, 1994), and to environmental stress during development (Leary and Allendorf, 1989).

Patterns of asymmetry with respect to age, sex and population are described for the two bilateral traits tarsus and wing length, and their interpretation is discussed.

5.4: The third approach is to explore to what extent the morphometric data collected in this study may be used to estimate body condition. Much attention has been paid to developing methods of assessing a birds body condition in terms of nutrient stores and reserves, from measurements obtainable from live birds in the field (Wishart, 1979; Piersma, 1984; Sibly et al., 1987; Ormerod and Tyler, 1990; van der Meer and Piersma, 1994), as this would aid interpretation of mortality and fecundity data in wild population studies, and may also have population management implications (see 5.4:1). This is followed by discussion of an alternative approach to assessing condition in birds: the analysis of a blood parameter (packed cell volume). The merits of the two methods are then compared.

The implications of these three approaches for conservation management are discussed in the final section: 5.5.

5.2: Assessing the suitability of morphometric traits for heritability estimates

5.2:1 Correlations among traits

Introduction

A high correlation among the traits tarsus length, wing length and weight would suggest that they were measures of a single trait which could be described as 'body size'. If this was the case, future studies could measure a single component of body size without a loss of information, and the handling time of each bird could therefore be reduced. In addition, if there was a high correlation between the left and right sides of the bilateral traits, the mean value could be used for subsequent analyses, and future studies could measure a single side, if symmetry itself was not the subject of investigation.

Many avian studies have used a single morphometric trait as a description of 'body size', often in the absence of a definition of body size, and without evidence that the chosen trait is a suitable predictor of any other traits. Weight, tarsus length and wing length are commonly used for this purpose, due to their ease of measurement on live birds. Karlsson et al. (1988) for instance, assessed the size of robins *Erithacus rubecula* by wing length; James (1970) discussed geographical size variation in 12 American bird species using wing length as a measure of size. Andersson (1993) measured sexual size dimorphism in Jackson's widowbird *Euplectes jacksoni* based on tarsus length; Marti (1990) cites several references in support of the use of weight as an indicator of body size in his study of sexual size dimorphism in the barn owl.

This approach is open to two main criticisms: firstly, studies which have examined correlations among a number of skeletal and external body components have found low correlations among traits. McGillivray (1989) took 18 skeletal measurements from a sample of 418 Great horned owls *Bubo virginianus* taken from populations across North America, and found more geographic variation in the size of body core variables such as keel length, than for skull, leg or wing characters; owls from northern regions had relatively larger body cores. Johnston (1990) found relative limb length to vary inversely with core size in pigeons, with larger pigeons having proportionally shorter wings and hence greater wing loading. The sexes may also vary in their body proportions; McGillivray (1985) found the greatest sexual dimorphism in tarsometatarsus length in Great horned owls; of the other skeletal measures, flight elements were more dimorphic than head or other leg measures; females were larger than the males in each case with the exception of skull width, where males had significantly wider skulls than females. These studies demonstrate that birds may vary in their skeletal proportions, with differences arrising according to sex and geographical location. A single skeletal measure may therefore be a poor predictor of other components, if these factors are not taken into account. Any attempt at simply describing 'skeletal size' using one or a few skeletal elements is therefore an oversimplification of actual patterns of skeletal size variation.

The second criticism of using a single trait to describe body size is that traits measured externally in birds are subject to different sources of variation (see 5.1); they often include non-skeletal components. Hence although tarsus length most closely approximates a skeletal measure, wing length includes feather growth, and weight is influenced by food and water intake, nutrient stores and reserves (Ormerod and Tyler, 1990). Seasonal and diurnal changes in these elements may therefore result in different estimates of 'size' according to the time of sampling.

A combination of these two factors- birds varying in their skeletal body proportions, and other sources of variation acting in externally measured traits, may result in low and non-significant correlations among morphometric traits obtainable from live birds. McGillivray (1985) reported low intercharacter correlations between external characters in Great horned owls; the Spearman rank correlation coefficients for weight and body length were 0.31** in females (n= 109) and 0.27 in males (n = 58); weight and wing length correlations were 0.23* in females and -0.13 in males. Taylor (1994) reported a total lack of significant correlations among external body measurements in his study of wild barn owls. The correlations among three morphometric traits are therefore calculated for the barn owls in this study, to test the hypothesis that there are no significant correlations among the traits.

Methods

(1) Correlations in bilateral traits

Pearsons correlation coefficients for the left and right sides of the bilateral traits wing and tarsus length were calculated for the barn owls sampled as described in chapter 3 and sexed as described in chapter 4. Birds with injured wings or tarsi were excluded, as for all the quantitative trait analyses. Data were included from both sexes, adults and juveniles, from the 5 study populations combined.

(2) Correlations among traits

Pearsons correlation coefficients were calculated for the three traits wing length, tarsus length and the cube root of weight; the weight data were converted to allow direct comparison with the linear measurements; the mean of left and right sides was used for the bilateral traits. Significance levels for the correlations were calculated as Bonferroni adjusted probabilities, to correct for the occurence of significant correlations arrising by chance where multiple correlations are computed (Wilkinson et al., 1992).

Correlations were confined to adult barn owls, to avoid the confusion introduced by sampling young birds before wing extension was completed (see chapter 3). Correlations were initially calculated on the sexes seperately, due to the possibility of the sexes differing in their body proportions (McGillivray, 1985). The data were then further sub-divided into the separate populations, and into wild and captive categories, and intertrait correlations computed for each sex and population category.

Results

(1) Correlations in bilateral traits

The correlation coefficients for the bilateral traits are presented in table 5.1. The regressions of right and left tarsus, and right and left wing length are illustrated in figs. 5.1 and 5.2.

Table 5.1 Correlations between right and left sides of bilateral traits of adult and juvenile barn owls, from the five populations combined. n = the number of barn owls sampled, r = the correlation coefficient, and t = the t value for the correlation, denoting the significance of the correlation between the two traits. Significance levels: ***P<0.001; **P<0.01; *P<0.05

trait	n	r	t
tarsus	208	0.854	23.560 ***
wing	211	0.993	125.652 ***

As the correlations between right and left sides were high, the data are combined as mean tarsus and mean wing length for subsequent analyses.

(2) Correlations among traits

Distributions of the data and the relationships between traits are displayed graphically as histograms and scatterplots for adult males (fig. 5.3) and adult females (fig. 5.4). Pearsons correlation coefficients for these data are shown in table 5.2 for adult males and females. No significant intertrait correlations were found in this sample of 69 adult males; in the sample of 97 females the correlation betwen the cube root of weight and tarsus length was low (0.28*) but weakly significant.

The correlation coefficients for the data sub-divided into sex and population classes are shown in table 5.3 for males and 5.4 for females. Only one inter-trait correlation was significant at the 0.05 level; this was the correlation between tarsus length and cube root of weight for adult females in population 4 ($r = 0.67$, $p = 0.019$ *).

Due to the smaller sample size in the captive populations compared to the wild populations (see tables 5.3 and 5.4) the data were then subdivided into wild and captive categories and intertrait correlations calculated for each sex. The correlation coefficients for the data sub-divided by sex and by captive/wild are shown in tables 5.5; here, a significant correlation ($r = 0.37$, $p = 0.007$ **) is shown between wing and tarsus length in wild adult females ($n = 66$). The correlation between the cube root of weight and tarsus length, which was significant in adult females, is not significant when this category is subdivided into captive and wild females.

Fig. 5.1 The correlation between right and left tarsus, including data from both sexes, adults and juveniles, from the five study populations combined. n = 208, correlation coefficient = 0.854

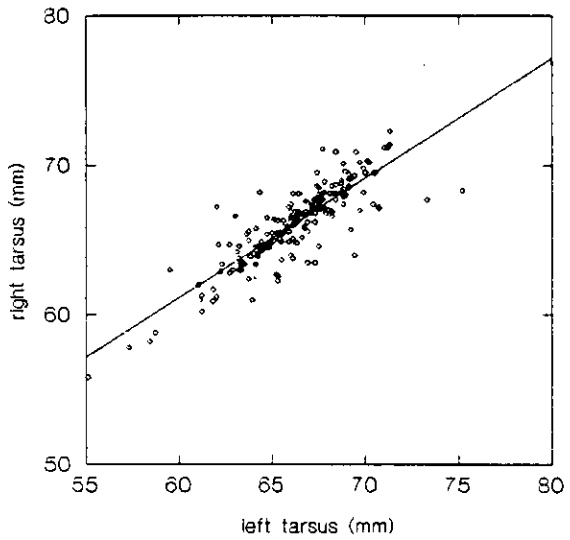


Fig. 5.2 The correlation between right and left wing length, including data from both sexes, adults and juveniles, from the five study populations combined. n = 211, correlation coefficient = 0.993

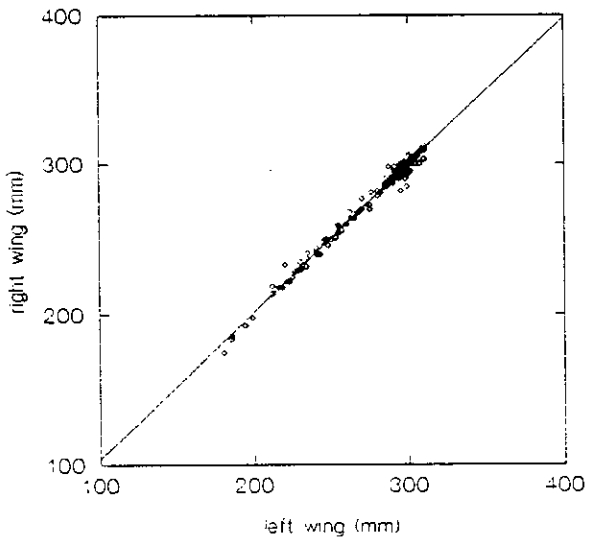


Fig. 5.3 Scatterplots and histograms for three morphometric traits (tarsus length, wing length and cube root of weight) for adult male barn owls (n = 69); all populations combined.

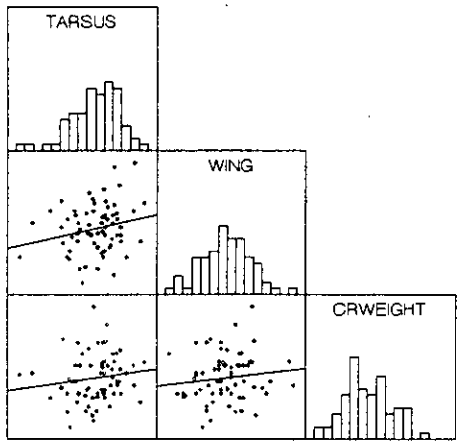


Fig. 5.4 Scatterplots and histograms for three morphometric traits (tarsus length, wing length and cube root of weight) for adult female barn owls (n = 97); all populations combined.

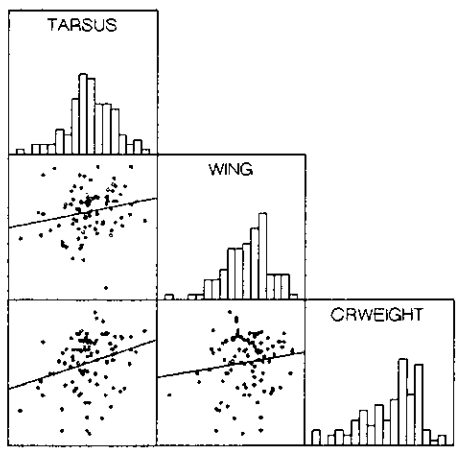


Table 5.2 Pearson correlation coefficients for the correlations shown in figs. 5.3 and 5.4 among tarsus length, wing length and cube root of weight for adult male and female barn owls; populations combined. p = Bonferroni adjusted probability.

TRAITS	MALES			FEMALES		
	n	r	p	n	r	p
tarsus/wing	69	0.222	0.202 ns	97	0.210	0.115 ns
tarsus/weight$^{1/3}$	69	0.145	0.702 ns	97	0.283	0.015 *
wing/weight$^{1/3}$	69	0.125	0.916 ns	97	0.141	0.504 ns

Table 5.3 Pearson correlation coefficients for the correlations among tarsus length, wing length and cube root of weight for adult male barn owls; populations analysed separately. p = Bonferroni adjusted probability.

Popn.	tarsus/wing			tarsus/weight$^{1/3}$			wing/weight$^{1/3}$		
	n	r	p	n	r	p	n	r	p
1	26	0.377	0.174 ns	26	0.164	1.000 ns	26	0.331	0.297 ns
2	21	0.095	1.000 ns	21	0.399	0.221 ns	21	0.210	1.000 ns
3	13	0.133	1.000 ns	13	0.092	1.000 ns	13	-0.111	1.000 ns
4	5	0.069	1.000 ns	5	0.593	0.875 ns	5	-0.343	1.000 ns
5	4	0.110	1.000 ns	4	-0.424	1.000 ns	4	-0.027	1.000 ns

Table 5.4 Pearson correlation coefficients for the correlations among tarsus length, wing length and cube root of weight for adult female barn owls; populations analysed separately. p = Bonferroni adjusted probability.

Popn.	tarsus/wing			tarsus/weight$^{1/3}$			wing/weight$^{1/3}$		
	n	r	p	n	r	p	n	r	p
1	37	0.354	0.095 ns	37	0.194	0.748 ns	37	0.158	1.000 ns
2	27	0.422	0.086 ns	27	0.202	0.935 ns	27	0.078	1.000 ns
3	14	0.370	0.580 ns	14	0.324	0.773 ns	14	0.499	0.209 ns
4	15	-0.036	1.000 ns	15	0.669	0.019 *	15	0.266	1.000 ns
5	4	-0.978	0.066 ns	4	-0.163	1.000 ns	4	0.189	1.000 ns

Table 5.5 Pearson correlation coefficients for correlations among tarsus length, wing length and cube root of weight for adult barn owl data sub-divided into sex and wild/captive categories. p = Bonferroni adjusted probability.

	TRAITS	MALES			FEMALES		
		n	r	p	n	r	p
WILD	tarsus/wing	49	0.321	0.074 ns	66	0.369	0.007 **
	tarsus/weight$^{1/3}$	49	0.232	0.327 ns	66	0.211	0.269 ns
	wing/weight$^{1/3}$	49	0.228	0.343 ns	66	0.055	1.000 ns
CAPTIVE	tarsus/wing	20	-0.032	1.000 ns	31	-0.038	1.000 ns
	tarsus/weight$^{1/3}$	20	0.237	0.941 ns	31	0.386	0.095 ns
	wing/weight$^{1/3}$	20	0.175	1.000 ns	31	0.345	0.173 ns

Discussion

The strong correlations between the two sides of the bilateral traits indicates that the data can be combined as the mean of both sides for subsequent analyses, and that in future studies, measuring a single side would be adequate.

Only three of the intertrait correlations computed were significant, and these indicated a low correlation between the traits. The weakly significant correlation between cube root of weight and tarsus length in adult females was not significant when the data were subdivided into wild and captive categories; this could be due to the reduced sample size of the subdivided samples. The significant correlation between wing and tarsus length in wild adult females was not reflected in the larger sample of females from all populations combined, however; this suggests a confounding effect to the correlation in the combined populations due to the captive adult females. Increased feather wear in captivity due to abraision against the enclosures, or the accidental inclusion of data from captive populations where the primary feathers were not fully extended after moult could account for this difference in intertrait correlations.

The general lack of significant correlations, and the low values of the significant correlations demonstrated in this data set show that each trait measured in this study is a poor predictor of the others. This result is in concordance with the low intertrait correlations found by McGillivray (1985) for Great horned owls; the presence of at least some significant correlations, however, brings Taylor's (1994) claim of a total lack of inter-trait correlations in barn owls into question; a larger data set allowing for the subdivision of the data more rigerously into sex, age, population, season etc. could remove confounding sources of variation and therefore improve the significance of the intertrait correlations.

Given the apparent low intertrait correlations for wing length, tarsus length and cube root of weight, the question then arises, can one of these external measures be chosen as the best estimator of total body size in birds? Many avian studies describe 'body size' in terms of the first principal component of a PCA performed on a large number of skeletal measures (e.g. McGillivray, 1985; Ibe, 1989; Rising and Somers, 1989; Freeman and Jackson, 1990; Merila and Gustafsson, 1993). Accepting this definition, the correlation between an externally measured trait and the PC1 gives an indication of how good the trait is as a predictor of body size. McGillivray (1985) found wing length to be the best external measure (40% correlation with PC1) in Great horned owls; Freeman and Jackson (1990) favoured tarsus or mass (40-60% correlation with PC1) in three passerine species. Rising and Somers (1989) considered tarsus to be the best estimator of body size in Savannah sparows *Passerculus sandwichensis*, not only because it showed the highest correlation with PC1 (r = 0.67-0.86 in females, 0.78-0.89 in males), but also was the trait least subject to temporal variation. The differences in the conclusions from these studies, and the low predictive ability of the best external trait in McGillivray's (1985) and in Freeman and Jacksons' (1990) studies, highlights the problems associated with the use of a single external trait to describe body size; interspecific generalisations on the optimum trait are not appropriate, and in any case the predictive ability of the external traits may be very low. It is not possible, therefore, to select one of the traits measured on this sample of barn owls as the best estimator of 'body size', in the absence of a detailed morphometric study of skeletal components in barn owls.

In conclusion, the intertrait correlations shown in this study indicate that the traits wing length, tarsus length and weight should not be considered as covarying components of a single trait, 'body size' in this sample of barn owls. In particular, heritability estimates (see chapter 6) should be specific to each trait, and it would be inappropriate to extrapolate to a discussion on the heritability of body size on the basis of these data.

5.2:2 Measurement error

Introduction

If more than one measurement of a trait may be obtained from each individual, the phenotypic variance may be partitioned into variance within and among individuals. The ratio of these variance components is termed the repeatability (r), which sets an upper limit to heritability estimates (see chapter 6) and can be taken as an assessment of measurement error (Falconer, 1981).

An assessment of measurement error is a valuable exercise at the outset of any study of quantitative traits; a preliminary study can identify those traits with high r values as suitable for further study, or alternatively can be used to determine what gain in accuracy would be obtained by multiple measurements of the trait.

Repeatability is commonly estimated either by repeated measurements of a particular trait over a period of time, or by measuring equivalent traits on an individual, such as characters that can be measured on two sides of the body. To give an estimate of measurement error, repeated measurements should be taken at short time intervals if the trait is expected to vary with time. Body weight may vary diurnally, for instance, and so repeatability measures should be taken within minutes of each other. For skeletal measures, which remain constant once an adult size has been attained, repeated measurements of adults could occur at any time.

Bilateral measurements are only suitable for estimates of measurement error if it is assumed that the organism is symmetrical, yet as described in section 5.3, departures from symmetry are a possible source of variation in quantitative traits. In this study, tarsus and wing length were measured for both sides of the body, but it was not considered justified to extend the handling time of the birds to do multiple measurements of these traits. Estimates of measurement error were therefore not obtained from the live barn owls sampled. An indication of the likely measurement error for the live birds was possible, however, by multiple measurements of a sample of dead barn owls.

5.2:2 Methods

Barn owls which had been found dead by members of the public, and sent to the Institute of Terrestrial Ecology, Huntingdon for analysis of pesticide residues (Newton et al., 1991) were available for measurement. As these birds had been dissected and stored frozen, repeated weight measurements were not appropriate, but tarsus and wing length could be measured from the thawed specimens. Measurements were confined to birds of one sex, to prevent possible sex differences increasing the among bird component of variance.

A sample of 10 adult female barn owls, selected as the least decomposed or desiccated of the specimens available, were individually labelled and measurements of right and left tarsus, and right and left wing length were taken, following the procedure described for the live birds in chapter 3. Each measurement was repeated 10 times, the order in which the birds were measured being selected at random. The time interval between measurements for each individual was a few minutes, depending on the order of selection. Each set of measurements

was recorded on a separate sheet, to reduce the likelihood of previously recorded measurements influencing a trial.

The differences in measurement error between live and dead barn owls were minimised by handling the dead specimens in the same manner as the live birds. Although this approach is open to criticism in that the source of measurement error may not be the same for live and dead birds, it was considered the most appropriate method for estimating measurement error given the constraints on this study.

Repeatability was calculated by ANOVA, where r is given by the intra-class correlation (t):

$$t = \sigma^2_b \; / \; \sigma^2_b + \sigma^2_w$$

σ^2_b = the variance among individuals; σ^2_w = the variance within individuals;

σ^2_b = (mean square among individuals - mean square within individuals) / n

The expected mean square for variance within individuals is given by the within individual mean square in the ANOVA; the expected mean square among individuals is:

$$\sigma^2_w + n\,\sigma^2_b$$

where n= number of measurements taken.
(after Snedecor and Cochran, 1980)

Results

(i) preliminary data description

The mean, standard deviation (SD) and coefficient of variation for each trait are presented in table 5.6. The statistics were calculated from the 10th set of measurements taken, to control for the possible source of error introduced by the measurement of specimens in different states: the specimens became more flexible with repeated handling.

Table 5.6 Summary of tarsus and wing lengths from a sample of 10 dead female barn owls.

trait (mm)	mean	SD	coefficient of variation
right tarsus	7.055	0.277	0.039
left tarsus	7.035	0.273	0.039
right wing	296.7	7.273	0.025
left wing	297.1	7.187	0.024

The variance of right and left measurements did not differ significantly ($F_{tarsus} = 1.013$, df = 9,9; $F_{wing} = 1.024$, df = 9,9), and no significant difference was found between the means of the right and left measurements of each trait (paired t test on right and left tarsus, t = 0.866, df = 9; paired t test on right and left wing, t = 0.352, df = 9).

(ii) Repeatability

Repeatability estimates are presented in table 5.7; all estimates are high, with wing measurements having slightly higher r values than the tarsus measurements.

Table 5.7 Repeatability estimates for tarsus and wing lengths of 10 dead barn owls, from 10 measurements per individual for each trait.

trait	repeatability
right tarsus	0.923
left tarsus	0.818
right wing	0.969
left wing	0.971

Discussion

As the means and variances of the two sides of each trait did not differ significantly, this suggests that the measurement error for the two sides would be similar, and not influenced, for instance, by the handedness of the measurer when manipulating the bird, callipers or wing rule.

The repeatability values are very high, indicating that measurement error is not a major source of variation in either wing or tarsus length; reference to Falconer (1981, fig. 8.3) shows that little gain in accuracy would be obtained by taking more than one measurement of these traits. It is assumed that similar values would be obtained from the same measurements carried out

on live birds, and therefore that the phenotypic values of a single measurement may be accepted in the confidence that the measurement error is small, and that the observed variance is due to other environmental or genetic factors. As far as measurement error is concerned, therefore, both tarsus length and wing length appear suitable candidates for heritability estimates, as the upper limit to heritability set by the repeatability is close to the maximum of 1.00 for each trait.

5.2:3 Patterns of variation

Introduction

Differences in metric traits according to sex, age or population would influence the method employed to estimate heritability and the interpretation of heritability results; if the trait was sexually dimorphic, for instance, heritability should be estimated for each sex separately. If differences occured between adults and juveniles, this difference should be taken into account in the analysis, or the age dependent traits could be avoided in heritability estimates based on data from adults and juveniles. The relative amounts of variation within and among populations, as described by the coefficient of variation, are of interest in assessing the value of estimating heritability for a particular trait.

Phenotypic variation in metric traits may be attributed to a number of different processes. Sexual dimorphism may be attributed to differences in skeletal structure between the sexes, for instance (McGillivray, 1985), or to differences in reproductive organs, fat or protein deposits, depending on which trait is quantified (Marti, 1990). Age differences may be attributed to normal developmental processes, but juveniles will not necessarily have lower mean values than adult barn owls for all traits, as their pre-flight weight may exceed the parental weight (Taylor, 1994). Population differences could be attributed to geographical size variation correlating with latitude (McGillivray, 1989), or may be due to the inclusion of unusual breeding stock in captivity, or to different environmental factors influencing growth and condition in the different populations. In this section, the metric traits are therefore analysed for sex, age and population differences.

Methods

Data were collected from birds sampled as described in chapter 3 and assigned a sex as described in chapter 4. Data were classed according to the following categories: captive/wild; male/female; adult/juvenile; population 1-5. Additional data also available included: month sampled; natal and sample sites of wild birds; year fledged and sampled, and age in years.

Preliminary analysis of patterns of variation was attempted by ANOVA. A maximum likelihood technique (REML) was then employed as a more powerful approach to analysing patterns of variation in data sets which are unbalanced in statistical design, and which allows greater flexibility in terms of the number of possible contributing factors that can be included in inter-group comparisons.

Summary statistics

Summary statistics for the traits weight, tarsus length and wing length are presented in tables 5.8-5.10; the data are sub-divided into sex, age and population classes. Comparing the coefficients of variation among traits, tarsus length appears to be less variable overall than weight or wing length, and values are similar for both adults and juveniles. Wing length, despite low CV in adults, has the highest CV in juvenile birds.

ANOVA

As can be seen from tables 5.8-5.10, these data are characterised by unequal sample sizes among the sex, stage and population categories, with some categories represented by a single individual, and with few observations overall for some populations. Such data can not be analysed for sex, age and population differences by a single fully factorial ANOVA, and so the preliminary analysis of patterns of variation was performed by Systat's 'general linear model' where interactions are only computed if specified in the model. Even so, the data from population 5 were insufficient to be analysed in this way, and from this and subsequent ANOVAs the data from population 5 were excluded from analysis. The preliminary ANOVA was therefore based on populations 1-4 nested within the captive/wild category, with sex and stage as additional factors. The interaction of sex and stage was also computed for each trait. The results of these ANOVA are shown in table 5.11.

TARSUS

No significant effects of age, sex, population or wild/captive were demonstrated for tarsus length, in the ANOVA based on 216 birds.

WING

From the preliminary ANOVA (table 5.11), adults were shown to have significantly longer wings than the juveniles by a mean of 47.74mm over all the populations analysed (populations 1-4). Captive barn owls had significantly longer wings than the wild birds sampled; a comparison of mean differences among populations showed that the mean wing length of birds in population 4 exceeded that of both wild populations by 10.20mm for population 1 ($p =$

0.027) and by 10.94mm for population 2 (p = 0.027). No other pairwise comparisons of wing length among populations was significant. To investigate patterns of variation in wing length further, an ANOVA was performed on adults among the four populations; no significant difference in adult wing lengths was revealed (n = 169, p = 0.217). A similar ANOVA for juveniles was based on populations 1 and 4 only due to sample sizes; mean wing length of juveniles in population 4 exceeded that of population 1 by 49.37mm (n = 54, p = 0.000). Age effects were then tested within populations; adult wing length exceeded that of juveniles by 48.49mm (n = 113, F = 216.584, p = 0.000) in population 1, but populations 2-5 could not be analysed in this way due to the small sample of juveniles per population. The data were then divided into captive and wild categories, and tested for age differences as before; there was a significant difference in wing length between adults and juveniles in each category; with wild adults exceeding wild juveniles by a mean of 51.26mm (n = 170, F = 397.056, p = 0.000), and captive adults exceeding captive juveniles by a mean of 14.15mm (n = 77, F = 12.161, p = 0.001).

WEIGHT

The preliminary ANOVA (table 5.11) showed significant effects of sex, stage and population (P1-P4); females exceeded males in weight overall by a mean of 50.01g, and juveniles exceeded adults in weight by 22.92g. The population difference, shown by the nesting result to be not attributable to a difference between captive and wild populations, was shown by pairwise mean comparisons (with Bonferroni adjusted probabilities) to be due to P1 exceeding P2 in weight by 19.46g (p = 0.008). No other pairwise population comparisons were significant.

Separate ANOVAs were performed within sex and age classes to further investigate patterns of variation in weight; Significant differences among populations were shown for adult males, where P3 exceeded both P1 and P2 in weight by means of 26.25g (p = 0.029) and 34.92g (p = 0.002) respectively, and for juvenile males, where P1 exceeded P4 in weight by a mean of 53.72g (p, = 0.042). No other population differences were significant in the case of the adult males; for the juvenile males, populations 2, 3 and 5 were excluded from the analysis due to sample size. No significant differences in weight were found among populations 1-4 for adult females; differences among populations for juvenile females could not be computed by ANOVA due to sample size.

Analysis of variation among sex and age classes within populations gave a significant sex effect in both adults and juveniles in population 1, by pairwise comparisons (with Bonferroni adjusted probabilities): adult females exceeded adult males in mean weight by 70.66g (p =

0.000), and juvenile females exceeded juvenile males by 36.31g ($p = 0.000$). A significant age effect in P1 was also shown for males, with juvenile males exceeding adult males by 48.46g ($p = 0.000$), but there was no significant age effect in females shown by this model. In population 2, there was insufficient data to allow a similar ANOVA for sex and age differences, but an ANOVA confined to adults showed a significant sex effect, with females exceeding males in weight by 56.38g ($n = 49$, $F = 28.06$, $p = 0.000$). Comparisons of sex and age effects in P3-P5 seperately were not possible by ANOVA.

Identifying and interpreting patterns of variation by ANOVA as described in this section is appropriate for specific hypothesis testing, particularly if the data set is large. Reference to tables 5.8-5.10, however, show that with this data set, many categories comprise data from a small number of individuals; in some cases data were available from only a single individual. Analysis of variance is not appropriate or feasible in such cases, and so in the analyses described above, certain categories were omitted during the computation of each test (e.g. population 5 data excluded from each ANOVA). A method of analysis which makes the optimum use of all the available data would clearly be preferable in studies of this nature; in the following section, the data are analysed by a more powerful method for which a balanced design is not required.

Table 5.8 Summary statistics for the trait WEIGHT (g); data sub-divided into sex, age and population classes. P = population, SD = standard deviation, CV = phenotypic coefficient of variation.

P	MALES				FEMALES			
	ADULT		JUVENILE		ADULT		JUVENILE	
	n	mean SD CV	n	mean SD CV	n	mean SD CV	n	mean SD CV
1	27	311.4 27.74 0.089	21	359.9 30.44 0.085	37	382.1 26.64 0.070	23	396.2 32.16 0.081
2	22	302.8 22.26 0.074	6	329.0 78.32 0.238	27	359.2 45.62 0.127	2	387.5 53.03 0.137
3	13	337.7 32.90 0.097	3	339.3 24.54 0.072	14	367.8 41.22 0.112	1	358.0
4	6	332.0 20.79 0.063	3	316.7 53.46 0.169	15	355.0 33.00 0.093	2	342.5 60.10 0.175
5	4	303.3 19.21 0.063	1	363.0	4	340.5 46.63 0.137	3	315.7 17.93 0.057

Table 5.9 Summary statistics for the trait TARSUS LENGTH (mm); data sub-divided into sex, age and population classes. P = population, SD = standard deviation, CV = phenotypic coefficient of variation.

P	MALES				FEMALES			
	ADULT		JUVENILE		ADULT		JUVENILE	
	n	mean SD CV	n	mean SD CV	n	mean SD CV	n	mean SD CV
1	27	66.47 2.14 0.032	17	67.05 1.62 0.024	39	66.85 2.04 0.031	20	66.04 2.03 0.031
2	21	66.97 2.16 0.032	6	63.61 2.99 0.047	27	66.41 2.73 0.041	2	67.27 4.14 0.061
3	13	66.80 3.27 0.049	3	66.15 1.65 0.025	14	65.61 2.80 0.043	1	63.40
4	6	65.88 2.35 0.036	3	66.33 4.27 0.064	15	66.92 3.16 0.047	2	66.80 4.95 0.074
5	4	64.90 4.53 0.070	1	65.75	4	65.86 2.44 0.037	3	60.98 4.88 0.080

Table 5.10 Summary statistics for the trait WING LENGTH (mm); data sub-divided into sex, age and population classes. P = population, SD = standard deviation, CV = phenotypic coefficient of variation.

P	MALES				FEMALES			
	ADULT		JUVENILE		ADULT		JUVENILE	
	n	mean SD CV	n	mean SD CV	n	mean SD CV	n	mean SD CV
1	28	295.5 5.59 0.019	19	249.5 31.78 0.127	39	294.9 6.80 0.023	21	243.3 22.53 0.093
2	22	296.7 5.72 0.019	6	227.6 27.62 0.121	27	296.2 6.48 0.022	2	243.8 22.98 0.094
3	13	295.7 7.52 0.025	3	233.8 26.38 0.113	14	297.1 5.65 0.019	1	240.5
4	5	295.2 0.91 0.003	3	296.0 7.55 0.026	15	294.8 7.00 0.024	2	297.5 3.54 0.012
5	4	291.5 6.34 0.022	1	310.0	4	299.9 7.62 0.025	3	291.7 4.91 0.017

Table 5.11 Analyses of variance to test for differences among sub-sets of data for the traits weight, tarsus and wing length, divided according to sex, age and population categories. Populations 1-5 are nested within the WILD (captive/wild) category.

DEP VAR: TARSUS N:216 MULTIPLE R: 0.129 SQUARED MULTIPLE R: 0.017

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
WILD	1.463	1	1.463	0.237	0.627 ns
POPN{WILD}	10.342	3	5.171	0.840	0.433 ns
SEX	0.853	1	0.853	0.139	0.710 ns
STAGE	13.506	1	13.506	2.193	0.140 ns
SEX*STAGE	0.032	1	0.032	0.005	0.943 ns
ERROR	1287.321	208	6.159		

DEP VAR: WING N: 220 MULTIPLE R: 0.806 SQUARED MULTIPLE R: 0.649

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
WILD	1180.103	1	1180.103	4.695	0.031*
POPN{WILD}	1461.075	3	730.538	2.906	0.057
SEX	42.915	1	42.915	0.171	0.680
STAGE	88178.764	1	88178.764	350.793	0.000***
SEX*STAGE	2.533	1	2.533	0.010	0.920
ERROR	53541.828	212	251.370		

DEP VAR: WEIGHT N:222 MULTIPLE R: 0.632 SQUARED MULTIPLE R: 0.399

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
WILD	13.850	1	13.850	0.011	0.916
POPN{WILD}	18745.100	3	9372.550	7.529	0.001**
SEX	94142.431	1	94142.431	75.621	0.000***
STAGE	20375.614	1	20375.614	16.367	0.000***
SEX*STAGE	2413.488	1	2413.488	1.939	0.165
ERROR	267658.259	214	1244.922		

Maximum likelihood: REML

Introduction

Where data are unbalanced and a number of possible sources of variation are quantified, Residual Maximum Likelihood (or Restricted Maximum Likelihood, REML) is an appropriate statistical technique for estimating the amounts of variation present and estimating the effects of certain factors on the data (Shaw, 1987). REML was developed as a powerful tool in the analysis of plant and animal experimental data, where it is commonly employed for heritability estimates and in quantifying the effects of experimental treatments. REML gives results equivalent to those produced by ANOVA if the data are balanced, but its use in analysing unbalanced data gives it far wider applications. Shaw (1987) discussed the use of maximum likelihood approaches to quantitative genetics of natural populations; other uses in natural populations include investigating variation in growth rates of yellow-eyed penguin chicks *Megadyptes antipodes* (Van Heezik, 1990), discriminating between British and Icelandic redshanks *Tringa totanus* on the basis of morphometrics (Summers et al., 1988), and estimating population numbers of the territorial Northern spotted owl *Strix occidentalis caurina* from day and night counts (Ward et al., 1991).

REML is used in this study to assess the influence of a number of possible factors on the quantitative traits analysed by ANOVA above.

Methods

The components of variation for random effects are first calculated by pooling all the information in the data relevant to each source of variation; the resultant component of variation is an estimate of the variability which would be observed if the variability was due to that effect only, presented as the average squared deviation from the mean. Random effects refer to conditions outside experimental control, such as the effects of different batches of a drug, sampling at different times, or environmental differences between experimental plots. In a wild population study, random effects might include the effects of different researchers taking measurements, the year or month of sampling or different localities for data collection.

If the aim is to identify relevant components of variation, a model including as many random effects as available is initially employed. Factors showing negligible effect can then be sequentially removed, until a list of factors which may be relevant is obtained. The significance of each remaining random effect can be determined by repeating the analysis with each factor removed in turn; the difference between the log. likelihood deviances with the factor present and removed approximates a chi-squared value with one degree of freedom.

REML then estimates the effects of treatments (fixed effects). In an experimental situation, treatments involve the experimentally controlled factors such as the doses of a drug; in non experimental situations, fixed effects could include factors such as sex or age. The means for each trait are weighted according to the effects of the sources of variation determined in the first part of the analysis. The standard errors associated with the estimation of the treatment means will vary in an unbalanced analysis, but are presented as an average SE for each treatment. Dividing the mean SE by the difference between the means gives an approximate t-statistic, but interpretation of these values is complicated by unknown degrees of freedom. (t-tests may be included in future versions of the program). Interactions between effects can be included in the analysis, if they are suspected to be relevant.

The barn owl morphometric data were analysed by REML, using the Genstat statistical package. Data were available for 10 factors which could be relevant to the analysis; they were divided into fixed effects:

- Sex (male or female)
- Age (in years)
- Stage (categorised as adult or juvenile)
- Capbred (whether reared in the wild or in captivity)

And random effects:

- Population (1-5)
- Site fledged (data from population 1 only, 22 natal sites)
- Site sampled (data from population 1 only, 31 sites)
- Year fledged
- Year sampled
- Month sampled

Interactions between sex and stage, and between population and month were included.

Results

In preliminary runs including all the factors described above, the differences in means for age were low in comparison with their average SE. As some of the information in this factor is in any case included in 'stage', this factor was dropped from subsequent runs. The results of the REML fixed effects are presented in table 5.12; fixed effect interactions are in table 5.13, and the chi-square approximations for the random effects are shown in table 5.14.

Within the fixed effects, sex is the most important factor in describing weight variance, but not for the other traits, in accordance with the results of ANOVA. This analysis highlights other sources of variation, however; stage has a relatively high t-value for wing length, but whether the birds were wild or captive bred may be relevant to both tarsus and wing length. In addition, the differences in means for the interaction results indicate that an interaction between sex and stage may be relevant to the variance in weight, but t values were low for other traits. Of the random effects, the site and year of sampling, and the interaction between population and month were not significant for any of the traits; population was highly significant for weight, whereas the month and site fledged were significant for wing length.

Table 5.12 REML Fixed effects for metric traits. The significance of the difference between the adjusted means for the three fixed effects sex, stage and wild/captive bred is given by the approximate t value, calculated as difference between the means / SE.

trait	factors	categories	adjusted mean	difference	SE	approx. t
weight	sex	male	335.4	28.5	5.15	5.53
		female	363.9			
	stage	juvenile	357.7	16.1	7.42	2.17
		adult	341.6			
	wild/captive bred	wild	347.1	5.1	10.68	0.48
		captive	352.2			
tarsus	sex	male	65.37	0.07	0.42	0.17
		female	65.30			
	stage	juvenile	64.79	1.09	0.59	1.85
		adult	65.88			
	wild/captive bred	wild	66.12	1.57	0.45	3.49
		captive	64.55			
wing	sex	male	282.3	4.6	2.11	2.18
		female	286.9			
	stage	juvenile	270.4	28.4	3.44	8.26
		adult	298.8			
	wild/captive bred	wild	268.6	32.0	4.58	6.99
		captive	300.6			

Table 5.13 REML interaction of two fixed effects: sex and stage. t is calculated as {[(a + d) - (b + c)] / 2} / 2, where a, b, c, and d are the adjusted mean values i.e. a = juvenile male, b = adult male, c = juvenile female, d = adult female

	adjusted means		average SE	approx. t
	juvenile	adult	of differences	
WEIGHT				
male	352.8	317.9	8.34	2.25
female	362.6	365.3		
TARSUS				
male	64.9	65.8	0.67	0.22
female	64.7	65.9		
WING				
male	266.3	298.4	3.64	-1.02
female	274.6	299.3		

Table 5.14 REML 'X^2' values for random effects. The REML analysis was repeated with each factor removed in turn; the difference between the log. likelihood deviances with the factor present or removed approximates a X^2 distribution with one degree of freedom. The shaded areas represent random effects which had negative components of variance in preliminary runs. They were therefore removed, and the analysis repeated without them to give the X^2 values shown.

factor	weight	tarsus length	wing length
population	9 ***		
month	2 ns		25 ***
population*month			
site fledged	1 ns		64 ***
site sampled			
year sampled			
year fledged	3 ns	2 ns	

Interpretation of ANOVA and REML results

None of the possible explanatory variables in the ANOVA for tarsus length had a significant effect; this result was supported by the REML, which in addition showed no significant results for any of the random effects included. Tarsus length in this study was therefore not sexually

dimorphic, nor were there differences between adults and juveniles at the age of sampling. This is in accordance with the findings of Taylor (1994), where a growth curve for tarsus length indicates this trait reaches adult size by 40 days after hatching. The ANOVA and REML results are also in accordance in showing no significant differences in tarsus length among populations; the low CVs shown in table 5.9 indicate an overall low level of variation in this trait.

Wing length was also shown to exhibit no sexual dimorphism by both methods of analysis, but ANOVA indicated a strong age effect; REML showed that the month sampled and the site from which they fledged were significant variables. These differences may all be explained by the nature of wing growth in juvenile barn owls; Taylor (1994) describes how wing growth continues up to around 60 days after hatching, as although skeletal growth is completed some 20 days earlier, feather extension may not be completed by the time the young birds fledge. In this study, sampling of nestlings did not occur when the young were precisely the same age (see chapter 3) hence the greater variance in juvenile data, and the difference in mean adult and juvenile data; the significant effects of month and site may be explained by differences in the average age of each brood at the time of sampling.

ANOVA showed significant differences in weight for age and sex classes, with juvenile weight exceeding adult weight, and females exceeding males; weight also differed betweeen populations. REML results were again in accordance with the ANOVA, with population the only significant random effect. The age effect is opposite to the trend described for wing length; in this case, young barn owls at the age sampled may exceed adult weight; juvenile weight actually decreases as they reach fledging age (Taylor, 1994). Sexual dimorphism may be due to possible differences in skeletal proportions between the sexes, or due to differences in the weight of reproductive organs, egg formation, or fat or protein stores and reserves as a result of the different roles in reproduction. Population differences in weight, significant between the two wild populations, are more difficult to interpret; such differences are sometimes attributed to differences in body condition. The use of weight data in estimating body condition is discussed in section 5.3.

In the above analyses, the weight data were strongly sexually dimorphic, but tarsus and wing length were not. As this appears to conflict with the sexual dimorphism in barn owls described by Marti (1990), sexual dimorphism in this study is described in more detail in the following section.

Sexual dimorphism

Introduction

Sexual size dimorphism, defined as the difference between the mean body size of adult males and females, occurs in most animal species (see Lovich and Gibbons, 1992, for references to reviews on invertebrates, fish, amphibians, reptiles, birds and mammals). In raptors, the female is commonly the larger sex; as this is contrary to the situation for the majority of birds, this is termed reverse sexual size dimorphism (RSD) (McGillivray, 1985).

Studies of RSD in birds typically assess body size by a single morphometric trait such as weight or wing length. The limitations of using a single trait to describe body size were described in 5.2:1; comparison of RSD among studies is therefore complicated by the use of different traits, which may not be good predictors of other size traits, and which are subject to different sources of variation. Studies of RSD in owls are reviewed below.

Interspecific trends in RSD in owls

Earhart and Johnson (1970) measured wing length and weight for museum specimens of 32 forms of north American owls. An absence of dimorphism in wing length was recorded for six of these, and two showed slight SD with males larger. The range in RSD for the remainder (using Storer's index- see below) was from 2.13 in Screech owls *Otus asio cineraceus* to 6.64 in Great horned owls *Bubo virginianus virginianus*. Weight showed greater dimorphism, with only one species in which the males were heavier. The range of RSD (for cube root of body weight) was from 1.86 in Screech owls *Otus asio mccallii*, to 10.92 in Great gray owls *Strix nebulosa nebulosa*.

As females had shorter wings in proportion to their body weight, Earhart and Johnson conclude that females would be less manoeuvrable than the males, due to an increased wing load, and relate this to foraging differences. They correlate the degree of RSD in their species with five dietary categories representing the range of diet from arthropods to vertebrates; diet was assessed from the stomach contents of the museum birds, and from generalisations within the literature. They found greatest RSD in species which are predators of vertebrates ($r= 0.504$, $p=0.005$ for cube root of weight, and $r=0.342$, $p=0.05$ for wing length) and suggest that this is due to niche partitioning between the sexes, explaining that inter-sexual competition would be highest in species which preyed upon large prey items of limited abundance. Lundberg (1986) expanded this idea, suggesting that shorter wing length in males gave them the agility to catch more avian prey. The stomach contents of 70 pygmy owls *Glaucidium gnoma* lend support to this theory, as females had eaten more mammals, and fewer birds, than the males (Earhart and Johnson, 1970). Barn owls *Tyto alba pratincola* are

seen as outliers on the dietary category- RSD correlation, with lower dimorphism in weight than would be predicted from the general trend.

Within genera, Earhart and Johnson found an increase in RSD in weight with increasing body weight. The relationship of greater RSD in larger species is potentially confounding to the diet hypothesis, as large owls tend to specialise on vertebrates. However, three small owl species which prey on a large proportion of vertebrates have greater RSD than would be predicted from their body weight alone, which Earhart and Johnson treat as supportive of the idea that differences in RSD are due to the increased competition involved in taking vertebrate prey. No such relationship was found for wing length, however, and it is conceivable that the RSD-weight correlation is due to females which are predators of vertebrates having a greater opportunity to accumulate fat reserves than females which rely on invertebrate food. Arguments to explain increased wing load in females in terms of different foraging techniques become irrelevant if their large size is because they are fat when incubating, as they would not normally forage at all during this period. Data on the proportions of body skeletal components and fat deposits are not available for most of these species, but McGillivray's study of body size in Great horned owls (1985) indicates relatively uniform body cores but high RSD in weight, suggestive that fat is a major contributor to RSD.

Mueller (1989) found a correlation between RSD in weight and average latitude of the breeding range in Europe in four out of five studies (n=10 -13 species in each study) of owls, and a correlation between RSD and wing length in only one of these, which was in concordance with the trend described by Lundberg (1986). Assuming that northerly species experience a more unpredictable food source than their southern counterparts, Lundberg explained the trend in RSD in terms of larger females being better equipped to endure fasting during incubation. This was criticised by Mueller, however, who maintained that the ability to accumulate fat reserves is independent of core size, and that smaller females would have the advantage due to their lower energy requirements. Mueller (1989) notes that latitude also correlates with the incidence of vertebrates in the diet, with more invertebrates featuring in the diet of southern species.

Intraspecific studies of RSD in owls

Mueller (1989) compared RSD in weight in populations of *Tyto alba, Athene noctua, Strix aluco* and *Asio otus* from several different countries e.g. Italy and Netherlands for the barn owl. No correlation between latitude and degree of RSD was found in any of these species, in contrast to the trend described for interspecific differences. Lundberg (1986) predicted from the theory of female starvation during incubation that RSD in weight would be correlated with

latitude within species as well as between species, but the absence of such a trend lends further support to a prey-type argument, as prey taken by a particular species does not vary with latitude to the extent that it does among species.

McGillivray (1989) found geographic variation in Great horned owl size, with the largest birds in Ontario, and the smallest in Texas and California, but found RSD to be constant across all regions. He concluded that RSD was a species specific phenomenon, and that studies to resolve the origin of RSD should concentrate on species-specific interactions within mated pairs.

This approach was taken by Hakkarainen and Korpimaki (1991) in an impressive study of 379 breeding pairs of Tengmalm's owl; RSD for weight, wing, tail and beak lengths were calculated for the period 1981-1990. Voles are the preferred prey of Tengmalm's owls, and in this population are subject to 4 yearly population fluctuations; in low vole years, the owls take a wider range of prey species, and extend their foraging range. RSD in this population could therefore be correlated not only with age of owls and time of breeding, but with different nutritional levels, allowing various hypotheses concerning RSD to be tested.

Hakkarainen and Korpimaki (1991) found significant correlations between breeding success in males (measured by number of fledged young) and both weight and tail length; in low vole years, light, long tailed males were more successful, whereas in high vole years the heavy, short tailed males fledged more young. They explain this in terms of foraging efficiency, where light males would have better flight performance, and suggest that light males would have greater lifetime reproductive success due to their ability to fledge offspring in poor years. Selection in favour of large males acted in good years, as they might then be expected to be better at obtaining good territories. In this study it is assumed that male weight is an indicator of overall body size, as it does not vary over the breeding season, whereas the females weight does change. No significant correlation for female winglength and reproductive success was found; female weight was taken as an indicator of male foraging efficiency, and therefore not used as diagnostic of overall female size.

Hakkarainen and Korpimaki (1991) postulated that the latitudinal trend in RSD, which Lundberg (1986) had explained in terms of large females surviving starvation during incubation, is due to selection favouring smaller males where prey is more unpredictable.

Marti (1990) tested the hypothesis that RSD is maintained by female choice of small mates on a population of barn owls, using both live birds (n= 278) and dead specimens (n=166) to

measure RSD for weight, body length, tarsus, foot, tail and wing length. Significant RSD was recorded for all the characters except wing and tail length; males had significantly lower wing loading (mass / wing surface area). Body size did not appear to be a factor in mate choice in 66 pairs from Utah (*T. a. pratincola*), and 93 pairs from France (*T. a. alba and T. a. guttata*), where body size was assessed by weight, and categorised as small, medium or large for comparison. Seasonal effects of weight were assessed by comparing weights at different stages of breeding; male weights did not vary seasonally, but female weights showed large changes, being heaviest at the onset of laying. Marti (1990) concluded that selection operated in favour of lower wing loading in males to improve foraging efficiency, as evidence for female choice was lacking.

In summary, RSD has been described for a number of owl species, including barn owls. RSD was reported for adult *T. a. pratincola* in Utah (Marti, 1990), for the traits weight, body length, and tarsus length, and it is sometimes assumed that a similar dimorphism occurs in the British *T. a. alba* population (e.g. Johnson, 1991). Taylor (1994) however, found no sexual size dimorphism in measures of live adult birds in his study other than for body weight. RSD in *T. a. alba* is therefore investigated in this study, for both wild and captive populations.

Quantifying RSD

Marti (1990) tests the significance of differences between the sexes in barn owls by the use of t-tests, an appropriate method to critically assess differences between the sexes where the variances of each sex are similar. More commonly, however, RSD is quantified by an index score which compares means, without consideration of the variance or distribution of data.

Indices used to quantify sexual dimorphism may be broadly divided into those that rely on some form of ratio, and those that are based on differences, such as subtracting male from female weight. Indices based on differences are potentially misleading, as the same score may be given to differences which are small in comparison to the actual size, and to differences which actually represent a large proportion of the overall size. This type of index has been used to assess sexual dimorphism in primates (Gaulin and Sailer, 1984), but is not commonly used. Indices involving ratios are more common, and there are several variants, reviewed by Lovich and Gibbons (1992).

Lovich and Gibbons (1992) suggest four criteria by which a dimorphism index may be assessed; they are:

(a) For ease of interpretation, the index should exhibit proper scaling, i.e. if the difference between the sexes is doubled, this should be reflected by a twofold increase in the index value.
(b) It should have high intuitive value; the magnitude of the dimorphism should be immediately apparent from the index value, without having to refer to the formula used to calculate it.
(c) It should exhibit directionality, a positive value signifying that a particular sex was the larger.
(d) It should be symmetric around a central value, preferably 0, at which point the sexes are equal in size.

The index most commonly used to assess RSD in raptors is that of Storer (Earhart and Johnson, 1970):

Storer's dimorphism index (ID) = 100 (mean size of female - mean size of male) / 0.5 (mean size of female + mean size of male).

Storer's index was criticised by Lovich and Gibbons on the grounds that it is not intuitive, and does not exhibit proper scaling; indeed none of the reviewed methods met all four criteria. Lovich and Gibbons (1992) proposed an alternative alternative index, which does meet the requirements; it is:

The mean size of the larger sex divided by the mean size of the smaller sex, with the value assigned as positive if the females are larger, and negative if the males are larger. 1.00 is then added to the value when males exceed females, and 1.00 is subtracted when females exceed males, to make the distribution symmetric around 0.00.

The relationship between the index values and the degree of sexual dimorphism is shown in fig. 5.5 for Storer's index, and in fig. 5.6 for Lovich and Gibbons' index. It can be seen that although Storer's index departs markedly from a linear relationship, this only occurs at levels of extreme dimorphism. In owls, the greatest recorded RSD occurs in weight, and in Earhart and Johnson's study (1970) of 32 North American species/subspecies the largest dimorphism in (cube root of) weight occurred in the Great gray owl *Strix nebulosa nebulosa*, with a Storer's index value of 10.92. On no occasion does the mean value for one sex equal or exceed twice the value of the other sex. For practical purposes, therefore, the relationship described by Storer's index may be thought of as linear, with a value of 0.00 when the sexes are equal, 66.67 when the female value is double that of the male's, and -66.67 when the male value is double that of the female.

With Lovich and Gibbons index, a value of 0.00 indicates equality in the sexes; 1.00 indicates the female value double that of the male, and -1.00 when the male value is double that of the female. Both indices are used in the following calculations of RSD, to allow comparison with other studies.

Methods

Barn owls were sexed according to their behaviour or plumage, as described in chapter 4. Differences in sexual dimorphism among populations and age classes were first assessed by F and t-tests, where t (df = n - 4) was estimated (e. g. comparing adults in P1 and P2) as:

(mean female P1 - mean male P1) - (mean female P2 - mean male P2) / $\sqrt{}$ (total variance / n-4)

In addition to testing for differences in the degree of sexual dimorphism among categories, differences among the population categories in the mean values for each trait were also assessed by ANOVA. In these calculations, population 5 was omitted from the analyses due to the small sample size of adults of each sex. Data from the different age and population categories were only combined in subsequent calculations of sexual dimorphism if there were no significant differences either among groups for (mean female - mean male), or in the mean values for each sex among categories, as dimorphism based on a ratio between the sexes (e.g. both Storer's and Lovich-Gibbons indices) is influenced by both the actual difference between the sexes, and according to whether the difference represents a small or large proportion of the overall size. On the basis of these analyses, it was decided whether to combine data from adults and juveniles, wild and captive populations, or even combine all data for a single estimate of sexual dimorphism for each trait.

In addition to the traits weight, tarsus and wing length, the cube root of weight was calculated, to allow direct comparisons with the linear measurements and with other studies (Marti, 1990).

In assessing sexual dimorphism within a category (population or age class), differences in variance between the sexes were tested by F tests for each trait, and differences between the means were then tested by unpaired 2-tailed t-tests. Where variances were unequal, a separate variance t-test was favoured (Wilkinson et al., 1992); this adjusts the degrees of freedom to account for unequal variances, hence fractional degrees of freedom are possible. Where the variances did not differ significantly, the pooled variance t-test was employed. Storer's and Lovich and Gibbons' indices were also calculated, for comparison with other studies.

Fig. 5.5 Storer's index for sexual dimorphism: the relationship between sexual dimorphism index (SDI) value and the degree of sexual dimorphism (after Lovich and Gibbons, 1992)

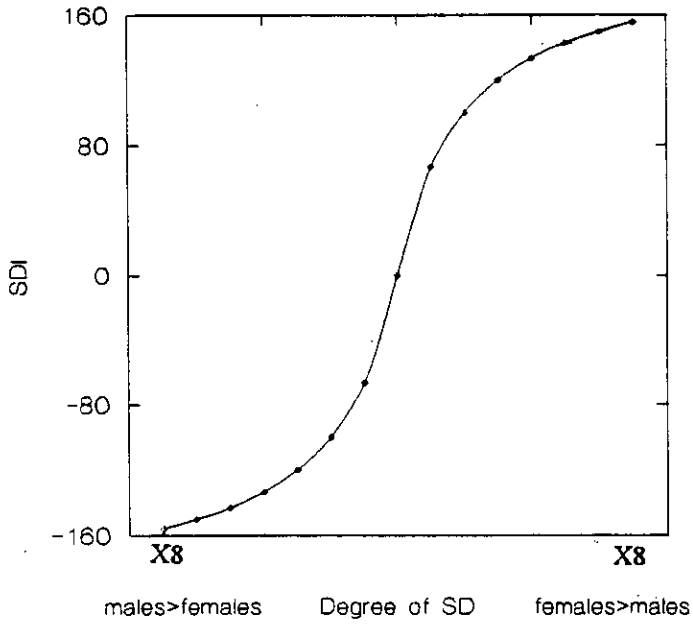
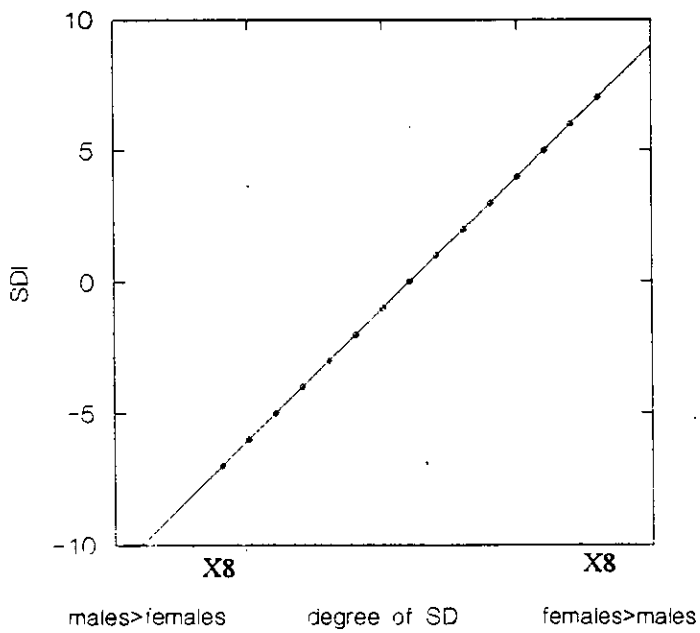


Fig. 5.6 Lovich and Gibbons' index for sexual dimorphism: the relationship between sexual dimorphism index (SDI) value and the degree of sexual dimorphism (after Lovich and Gibbons, 1992)



Results

(1) Preliminary analysis

WEIGHT

Preliminary analysis of the weight data showed a highly significant difference in (mean female-mean male) data between adults and juveniles in population 1, with adults exhibiting the greater degree of difference between the sexes (table 5.15). Adults in populations 1 and 2 also differed significantly in this parameter, as did all but one of the six possible pairwise comparisons of wild+captive populations, yet there were no significant differences within the captive populations. Table 5.16 shows the results of an ANOVA to test for differences among populations in adult male weight; the significant effect is shown in table 5.17 to be attributable to differences between populations 1+3 and 2+3, i.e. between two comparisons of a wild plus a captive population. No significant interpopulation differences in adult weight were found in the pairwise comparisons of adult females (tables 5.18-5.19), despite the significant effect of population in the preliminary ANOVA. On the basis of these analyses, it was decided that sexual dimorphism in weight should be assessed for adults and juveniles separately, and that data from the two wild populations should not be combined, but data from the three captive populations could be combined to increase the sample size for sexual dimorphism estimates for this trait.

TARSUS

The preliminary analysis of the tarsus data is shown in table 5.20. As with weight, tarsus showed a significant difference in (mean female-mean male) between adults and juveniles; significant differences were also shown within captive populations 3+4 and in wild/captive populations 1+3 and 2+4, but not between the two wild populations. The ANOVAs of tarsus length among populations 1-4 for each sex (tables 5.21 and 5.22) showed no significant differences among populations. On the basis of these analyses, it was decided that sexual dimorphism in tarsus should be asessed for adults and juveniles separately, and that data from the three captive populations should not be combined, but data from the two wild populations could be combined to increase the sample size for sexual dimorphism estimates.

WING

Table 5.23 shows the preliminary analysis of the wing data. Wing length of adults and juveniles differed in variance, and significant mean differences occured within captive and wild/captive comparisons, but not between the two wild populations. As for tarsus length, therefore, subsequent analysis of sexual dimorphism in wing length keeps adults and juveniles separate, and the three captive populations are not combined, but data from the two wild populations are pooled to increase the sample size.

Table 5.15 Differences in sexual dimorphism in WEIGHT among age and population categories. Diferences in variance assessed by F tests, where F = the greater variance in (females-males) / lesser variance in (females-males). Mean differences asessed by t-tests; df = N-4.

			F	df_1	df_2	sig.	t	df	sig.	RSD	diff.
Age	Adult	Juv.	1.325	42	62	ns	5.981	104	***	34.4	A>J
Within wild	P1	P2	1.742	47	62	*	2.344	109	*	14.3	1>2
Within captive	P3	P4	1.828	25	19	ns	0.718	44	ns	7.1	3>4
	P3	P5	1.094	25	6	ns	0.542	31	ns	7.1	5>3
	P4	P5	1.672	6	19	ns	1.114	25	ns	14.2	5>4
Wild/captive	P1	P3	1.880	25	62	ns	5.800	87	***	40.6	1>3
	P1	P4	1.028	19	62	ns	7.837	81	***	47.7	1>4
	P1	P5	1.719	6	62	ns	4.356	68	***	33.5	1>5
	P2	P3	1.080	25	47	ns	3.049	72	**	26.3	2>3
	P2	P4	1.694	47	19	ns	4.239	66	***	33.4	2>4
	P2	P5	1.013	47	6	ns	1.953	53	ns	19.2	2>5

Table 5.16 ANOVA to test for population differences in WEIGHT for adult males.

N:68	MULTIPLE R: 0.457		SQUARED MULTIPLE R: 0.209		
SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
POPN	12043.642	3	4014.547	5.640	0.002 **
ERROR	45557.300	64	711.833		

Table 5.17 Population differences in male WEIGHT. Pairwise mean differences and Bonferroni adjusted probabilities based on the ANOVA in table 5.16.

	POPNS	N	MEAN DIFF.	DIRECTION	PROBABILITY
Within wild	P1, P2	27, 22	8.672	1>2	1.000 ns
Within	P3, P4	13, 6	5.692	3>4	1.000 ns
captive	P3, P5	13, 4	-	-	-
	P4, P5	6, 4	-	-	-
Wild/captive	P1, P3	27, 13	26.248	3>1	0.029*
	P1, P4	27, 6	20.556	4>1	0.556 ns
	P1, P5	27, 4	-	-	-
	P2, P3	22, 13	34.920	3>2	0.002**
	P2, P4	22, 6	29.227	4>2	0.122 ns
	P2, P5	22, 4	-	-	-

Table 5.18 ANOVA to test for population differences in WEIGHT for adult females.

N: 93	MULTIPLE R: 0.304		SQUARED MULTIPLE R: 0.093		
SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
POPN	11937.980	3	3979.327	3.027	0.034 *
ERROR	117011.332	89	1314.734		

Table 5.19 Population differences in female WEIGHT. Pairwise mean differences and Bonferroni adjusted probabilities based on the ANOVA in table 5.18.

	POPNS	N	MEAN DIFF.	DIRECTION	PROBABILITY
Within wild	P1, P2	37, 27	22.960	1>2	0.085 ns
Within	P3, P4	14, 15	12.786	3>4	1.000 ns
captive	P3, P5	14, 4	-	-	-
	P4, P5	15, 4	-	-	-
Wild/captive	P1, P3	37, 14	14.322	1>3	1.000 ns
	P1, P4	37, 15	27.108	1>4	0.099 ns
	P1, P5	37, 4	-	-	-
	P2, P3	27, 14	8.638	3>2	1.000 ns
	P2, P4	27, 15	4.148	2>4	1.000 ns
	P2, P5	27, 4	-	-	-

Table 5.20 Differences in sexual dimorphism in TARSUS among age and population categories. Diferences in variance assessed by F tests, where F = the greater variance in (females-males) / lesser variance in (females-males). Mean differences asessed by t-tests; df = N-4.

			F	df_1	df_2	sig.	t	df	sig.	RSD	diff.
Age	Adult	Juv	1.296	64	35	ns	2.731	99	**	1.08	J>A
Within wild	P1	P2	1.910	46	64	ns	1.955	110	ns	0.94	2>1
Within captive	P3	P4	1.195	25	19	ns	2.535	44	*	2.23	3>4
	P3	P5	1.429	6	25	ns	1.784	31	ns	2.15	3>5
	P4	P5	1.707	6	19	ns	0.057	21	ns	0.08	4>5
Wild/captive	P1	P3	2.120	25	64	**	2.836	89	**	1.57	3>1
	P1	P4	1.774	19	64	*	1.221	83	ns	0.66	4>1
	P1	P5	3.028	6	64	*	0.894	70	ns	0.58	5>1
	P2	P3	1.110	25	46	ns	0.894	71	ns	0.63	3>2
	P2	P4	1.077	46	19	ns	2.273	65	*	1.60	4>2
	P2	P5	1.586	6	46	ns	1.668	52	ns	1.52	5>2

Table 5.21 ANOVA to test for population differences in TARSUS for adult males.

N:67 MULTIPLE R: 0.135 SQUARED MULTIPLE R: 0.018

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
POPN	6.802	3	2.267	0.388	0.762 ns
ERROR	368.501	63	5.849		

Table 5.22 ANOVA to test for population differences in TARSUS for adult females.

N:95 MULTIPLE R: 0.173 SQUARED MULTIPLE R: 0.030

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
POPN	18.348	3	6.116	0.936	0.427 ns
ERROR	594.576	91	6.534		

Table 5.23 Differences in sexual dimorphism in WING among age and population categories. Diferences in variance assessed by F tests, where F = the greater variance in (females-males) / lesser variance in (females-males). Mean differences asessed by t-tests; df = N-4.

			F	df_1	df_2	sig	t	df	sig	RSD	diff.
Age	Adult	Juv.	19.585	38	65	***	1.423	103	ns	5.6	J>A
Within wild	P1	P2	1.037	65	47	ns	0.086	112	ns	0.1	1>2
Within captive	P3	P4	1.776	25	18	ns	1.004	43	ns	1.8	3>4
	P3	P5	1.111	6	52	ns	3.901	58	***	7.0	5>3
	P4	P5	1.972	6	18	ns	3.543	24	**	8.8	5>4
Wild/captive	P1	P3	1.142	25	65	ns	1.473	90	ns	2.0	3>1
	P1	P4	1.555	65	18	ns	0.162	83	ns	0.2	1>4
	P1	P5	1.269	6	65	ns	5.722	71	***	9.0	5>1
	P2	P3	1.184	25	47	ns	1.262	72	ns	1.9	3>2
	P2	P4	1.499	47	18	ns	0.072	65	ns	0.1	2>4
	P2	P5	1.315	6	47	ns	4.927	53	***	8.9	5>2

Table 5.24 ANOVA to test for population differences in WING for adult males.

N:68 MULTIPLE R: 0.103 SQUARED MULTIPLE R: 0.011

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
POPN	23.603	3	7.868	0.228	0.877 ns
ERROR	2212.867	64	34.576		

Table 5.25 ANOVA to test for population differences in WING for adult females.

N:95 MULTIPLE R: 0.134 SQUARED MULTIPLE R : 0.018

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
POPN	72.114	3	24.038	0.554	0.647 ns
ERROR	3947.007	91	43.374		

(2) Sexual dimorphism

Sexual dimorphism for juvenile barn owls from population 1 is shown in table 5.26, and for adults from the 5 populations analysed separately in table 5.27. Significant differences between the sexes only occur for weight (and cube root weight) in any population or age class; the greatest difference is seen for adults of population 1, where females exceed males in weight by 70.66g, giving the greatest Storer's index score of 20.38. Females also exceed males in weight in population 2, but none of the differences between the sexes for populations 3-5 are significant. Juvenile females also exceeded males in weight in population 1, by a mean difference of 36.31g. When the three captive populations are combined for the weight data (table 5.28), however, the difference between the sexes is significant, with females exceeding males in mean weight by 28.5g, and a Storer's index of 8.26. Comparing the extent of sexual dimorphism in weight between the captive birds and the two wild populations showed the dimorphism to be significantly greater in the two wild populations (P1+captive populations combined: t = 7.338, df 116 ***; P2+captive populations combined: t = 3.994, df 101 ***).

Table 5.26 Sexual dimorphism in juvenile barn owls from population 1 tested by F-tests to compare variances, and t-tests to compare means; Storer's and Lovich-Gibbons indices for sexual dimorphism are included for comparison. df_1= degrees of freedom for sex with the largest variance, df_2= degrees of freedom for the sex with the smallest variance, ***P<0.001; **P<0.01; *P<0.05; not significant (ns) P>0.05. f>m = mean female exceeds mean male value.

trait	F	df_1	df_2	sig	t	df	p	mean diff.	Storer's index	Lovich-Gibbons index
weight	1.116	22	20	ns	3.838	42	0.000 ***	36.31 f>m	9.605	0.100
weight$^{1/3}$	1.051	20	22	ns	3.824	42	0.000 ***	0.232 f>m	3.212	0.033
tarsus	1.573	19	16	ns	1.650	35	0.108 ns	1.000 m>f	-1.516	-0.015
wing	1.990	18	20	ns	0.725	38	0.473 ns	6.267 m>f	-2.543	-0.026

Table 5.27 Sexual dimorphism in adult barn owls from wild and captive populations tested by F-tests to compare variances, and t-tests to compare means; Storer's and Lovich-Gibbons indices for sexual dimorphism are included for comparison. Populations 1 and 2 are wild barn owls,3-5 are captive. df_1= degrees of freedom for sex with the largest variance, df_2= degrees of freedom for the sex with the smallest variance, ***P<0.001; **P<0.01; *P<0.05; not significant (ns) P>0.05. f>m = mean female exceeds mean male value.

group	trait	F	df_1	df_2	sig	t	df	p	mean diff.	Storer's index	Lovich-Gibbons index
Popn. 1	weight	1.084	26	36	ns	10.299	62	0.000 ***	70.66 f>m	20.377	0.227
	weight$^{1/3}$	1.379	26	36	ns	10.323	62	0.000 ***	0.48 f>m	6.844	0.071
	tarsus	1.099	26	38	ns	0.728	64	0.469 ns	0.38 f>m	0.570	0.006
	wing	1.480	38	27	ns	0.418	65	0.678 ns	0.65 m>f	-0.222	-0.002
Popn. 2	weight	4.120	26	21	**	5.648	39.3	0.000 ***	56.37 f>m	17.033	0.186
	weight$^{1/3}$	3.592	26	21	**	5.544	40.9	0.000 ***	0.38 f>m	5.563	0.057
	tarsus	1.598	26	20	ns	0.763	46	0.499 ns	0.56 m>f	-0.834	-0.008
	wing	1.282	26	21	ns	0.341	47	0.735 ns	0.60 m>f	-0.203	-0.002
Popn.3	weight	1.570	13	12	ns	2.086	25	0.047 ns	30.09 f>m	8.531	0.089
	weight$^{1/3}$	1.551	13	12	ns	2.050	25	0.051 ns	0.20 f>m	2.806	0.028
	tarsus	1.362	12	13	ns	1.015	25	0.320 ns	1.19 m>f	-1.791	-0.018
	wing	1.775	12	13	ns	0.570	25	0.573 ns	1.45 f>m	0.490	0.005
Popn. 4	weight	2.521	14	5	ns	1.573	19	0.123 ns	23.0 f>m	6.696	0.693
	weight$^{1/3}$	2.238	14	5	ns	1.583	19	0.130 ns	0.15 f>m	2.186	0.022
	tarsus	1.807	14	5	ns	0.725	19	0.477 ns	1.04 f>m	1.566	0.016
	wing	59.34	14	4	**	0.234	15.3	0.818 ns	0.43 m>f	-0.147	-0.002
Popn.5	weight	5.894	3	3	ns	1.477	6	0.190 ns	37.25 f>m	11.573	0.123
	weight$^{1/3}$	5.000	3	3	ns	1.474	6	0.191 ns	0.256 f>m	3.740	0.038
	tarsus	3.450	3	3	ns	0.374	6	0.721 ns	0.963 f>m	1.473	0.015
	wing	1.446	3	3	ns	1.690	6	0.142 ns	8.375 f>m	2.832	0.029

Table 5.28 Sexual dimorphism in adult barn owls; data are combined in wild and captive categories where no significant differences among populations in (mean female-mean male) were detected by F or t-tests (see tables 5.15-5.23). F-tests compare variances, and t-tests compare means; Storer's and Lovich-Gibbons indices for sexual dimorphism are included for comparison.

	trait	F	df_1	df_2	sig	t	df	p	mean diff.	Storer's index	Lovich-Gibbons
Wild adults	weight										
	$weight^{1/3}$										
	tarsus	1.193	65	47	ns	0.039	112	0.969 ns	0.02 m>f	-0.024	0.000
	wing	1.398	65	49	ns	0.575	114	0.576 ns	0.67 m>f	-0.227	-0.003
Captive-bred adults	weight	1.608	32	22	ns	2.986	54	0.004 **	28.5 f>m	8.260	0.086
	$weight^{1/3}$	1.529	32	22	ns	2.978	54	0.004 **	0.14 f>m	2.714	0.028
	tarsus										
	wing										

RSD Discussion

The only significant differences in body size variables between the sexes in this study concerned weight. The large dimorphism in wild breeding birds suggests that the difference is due to the sexes different roles in reproduction; males are below their non-breeding weight due to the exertion of provisioning food for their mate and offspring, whereas the female lays down fat deposits and increases in weight due to egg production at this time. RSD for weight was also significant in the captive birds and in juveniles, however; this may reflect true differences in core body size between the sexes which are accentuated during breeding, although it is possible that the captive birds could have been approaching breeding condition at the time of sampling.

Marti (1990) reported a Storer's SD of 6.20 for cube root of weight in breeding pairs of *T. a. pratincola* in Utah, comparable to the 6.84 and 5.56 of the wild populations in this study. Marti (1990), however, also reported significant RSD in tarsus length, which was not apparent in *T. a. alba* in this study. Given that the dimorphism in weight, unlike that of tarsus length, may be attributable to non-skeletal components of the body such as fat, these results suggest that *T. a. alba* is less sexually dimorphic in size than the American sub-species. *T. a. pratincola* is larger overall than the British barn owl (Taylor, 1994); the apparent lack of RSD in tarsus length in *T. a. alba* is in accordance with the trend described by Earhart and Johnson (1970), in which smaller sub-species of owls exhibit less RSD than larger sub-species within a given species.

5.2:4 Implications for heritability analysis

The analyses in section 5.2 allow an assessment of how suitable the traits tarsus length, wing length, and weight would be for estimates of heritability in this study. The strong correlation between the two sides in the bilateral traits allows the data from the two sides to be combined as mean values for subsequent analysis, and the high repeatability of tarsus and wing length suggests that a single measurement is adequate for these traits. The low and non-significant intercorrelations between the three traits indicate that these traits should be dealt with separately in heritability estimates; they are not covarying components of a single trait such as 'body size'. On the basis of the repeatability estimates, both tarsus and wing length are suitable for heritability estimates as the upper limit to heritability set by the measurement error is close to the maximum of 1.00 in each case. The measurement error of weight was not assessed, but is also likely to be low.

Significant sexual dimorphism was recorded only for weight, and so for this trait heritability should be estimated for the sexes separately. Weight also showed a significant age effect; as skeletal growth in the juveniles was complete at the time of sampling, the difference may be attributed to differences in body condition and reproductive state between adults and juveniles. This is a source of error in heritability estimates for weight based on samples of parents and offspring; differences in variance between the adult and juvenile data sets can be corrected for in the heritability estimates, as described in chapter 6. The relatively high coefficient of variation for the weight data suggests that this trait exhibits sufficient variability to indicate a heritability study worth pursuing.

Wing length in adults exhibited a low coefficient of variation, in contrast to that of the juvenile birds. Variance was high in the juveniles as the trait was sampled before adult size was attained, and sampling did not occur at constant chick age. The combination of low levels of variation in the adults, and inflated variance in the juvenile birds suggests that this trait would not be suitable for heritability estimates.

Tarsus length exhibited intermediate levels of variability, as assessed by the phenotypic coefficient of variation, in comparison to the data from adults for the other traits. This trait was not sexually dimorphic, nor were there age differences in tarsus length for the barn owls sampled, and so heritability estimates could include juveniles of unknown sex, and no correction for differences between adult and juvenile variances would be required. On the basis of the analyses in this section, tarsus length would be suitable for heritability analysis. Heritability is estimated for weight and tarsus length in chapter 6.

5.3 Fluctuating Asymmetry

5.3:1 Introduction

Perfect symmetry is the phenotypic optimum for the majority of bilateral traits, particularly for characters such as wing or tarsus length, where departures from symmetry would impair the efficiency of locomotion. Measuring an individual's departure from symmetry in bilateral traits has been described by Polak and Trivers (1994) as possibly the best method available for assessing phenotypic quality, as with other methods, the optimum phenotypic state remains unknown. Symmetry has been shown to be an important factor in female choice, in experiments with coloured leg bands on zebra finches *Taeniopygia guttata*, and in a manipulated wild population of barn swallows *Hirundo rustica* (Hardy, 1994; Moller, 1993), indicating that assessing phenotypic quality from asymmetry may even be a naturally occurring phenomenon. A reliable method of assessing phenotypic quality would be a useful addition to the tools available for the management of captive breeding projects or for monitoring endangered wild populations.

Asymmetry is usually measured on individuals as the difference between right and left sides, and is described in terms of a population distribution. Where the differences between the two sides follow a normal distribution about a mean of zero (perfect symmetry), the phenomenon is termed fluctuating asymmetry. This distinguishes FA from two other types of bilateral symmetry; directional symmetry, where the mean is not zero, and antisymmetry, where symmetrical individuals are uncommon (Polak and Trivers, 1994).

As the same set of genes codes for both sides of a bilateral trait, departures from symmetry are ultimately environmental in origin. The degree of FA reflects the organisms ability to compensate for environmental perturbations during growth, i.e. is a measure of developmental instability; development is most stable where the genes have coevolved such that the regulatory mechanisms of growth are fine tuned against the effects of environmental stress.

The observed FA is therefore the product of both the magnitude of the environmental stress and the robustness of the genome to compensate for this stress. An increase in FA may be observed in association with an unusual increase in environmental stress during development, such as a parasite infection, or exposure to pesticides or other toxins (Leary and Allendorf, 1989). Conversely, disruption of a coadapted genome may be associated with an increase in FA in the presence of normal levels of environmental stress. Hence high FA has been correlated with inbreeding and a loss of heterozygosity, and with hybrids between two formerly isolated populations, in individuals with chromosome disorders, and where selection

has favoured a previously low frequency allele (Polak and Trivers, 1994); all these cases represent disruptions of established genomes. Studies which show a lack of correlation between FA and heterozygosity (Fowler and Whitlock, 1994) or any other of the correlates described above serve as a reminder that FA is an indicator of developmental instability irrespective of whether the cause is augmented environmental stress or a diminished ability of the genome to compensate for environmental influences on development. In addition, FA in one trait may not correlate with FA in another trait, if they differ in their timing or pathway of development; correlations between FA and the factors described above are the result of particular genetic and environmental circumstances, and should not be expected to be universally applicable. The differences in the sources of variation in barn owl tarsus length and wing length described in section 5.1, for instance, suggest that these traits would not necessarily exhibit the same pattern of variation in FA; FA of tarsus reflects skeletal development in the first 40 days after hatching, whereas variation in wing FA is influenced by feather growth, moult and wear in addition to skeletal structure. These differences should be considered in the interpretation of FA data.

Leary and Allendorph (1989) suggest that FA is a good measure of stress in captive and wild populations, arguing that as captive populations tend to experience a fairly constant environment, high FA values in comparison to a con-specific wild population are more likely to be indicators of a loss of genetic variation than increased environmental stress.

FA is therefore estimated for wild and captive barn owl populations in this study, to investigate population differences in FA in tarsus and wing length.

5.3:2 Methods

Barn owls sampled

Measurements of right and left tarsus and right and left wing length were taken from both adult and juvenile barn owls; two wild and three captive populations were sampled. The details of the catching and measuring of the birds, and descriptions of the populations, are presented in chapter 3. A small number of the barn owls held in captivity were wild, disabled birds which had suffered broken wings or tarsi as a result of traffic collisions. The tarsus and wing lengths of these birds were omitted from this and all subsequent analyses, as were the wing measurements of any adults with the relevant primary feathers incompletely grown, due to recent moult.

Estimating fluctuating asymmetry (FA)

Departures from symmetry are usually small, and so measurement error may be a large proportion of the within individual variance of a trait (Palmer and Strobeck, 1986). It is assumed that in this study, measurement error was small (see 5.2) and consistent across sex and age classes, and among different populations, and that comparisons of FA among these groups are therefore valid.

Palmer and Strobeck (1986) review 22 different indices for estimating FA, some of which correct for differences in character size, either at the level of the individual or the population, and some of which do not. The initial step in identifying an appropriate index is to test for size dependence of FA within samples; typically FA increases as character size increases. This can be tested by plotting a measure of FA for each individual, (R-L) against a measure of character size, such as (R+L)/2, where R = size of right side, L = size of left side. A greater spread of individual FA values as size increases indicates a scaling effect. This is illustrated in fig. 5.7 for tarsus measurements, and fig. 5.8 for wing lengths, where in each case, barn owls from all populations, sexes and age classes are combined. The distribution of FA with respect to size appears random for tarsus length, but with wing length, an increase in the spread of FA towards the larger wing lengths indicates a positive scaling effect.

An index uncorrected for size is therefore used to describe the individual tarsus FA values:

$$FA_{tarsus} = \text{var. } (R-L)$$

(i.e. the FA index is the variance of the difference between right and left sides)

Whereas for the wing measurements, a correction for trait size is included in the FA index:

$$FA_{wing} = \text{var. } [(R-L) / ((R+L) /2)]$$

(After Palmer and Strobeck, 1986).

FA can then be compared among sub-sets of the data by F-tests, if the data are normally distributed; it can be seen from the distributions of individual FA values for each trait in figs. 5.9 and 5.10 that this is appropriate for these data.

In this study, where no replicate measurements were taken, the degrees of freedom are given by (s-1)(j-1), where s= the number of sides measured i.e. 2, and j= the number of 'genotypes' i.e. the number of individuals sampled (Palmer and Strobeck, 1986).

5.3:3 Results

FA index values for the sub-sets of data are presented in table 5.29.

Sex differences in FA

The data were first tested for differences between the sexes, using adults from population 1. No significant difference in FA occurred between males and females in this sample (F_{tarsus}=1.6122, df= 37,22; F_{wing} =1.3302, df= 23, 37), and so sexes were combined for subsequent tests.

Age differences in FA

No significant difference in FA_{tarsus} was found in population 1 between young sampled in the nest and adults (F_{tarsus} =1.2748, df= 62, 42), but young birds had significantly greater FA_{wing} than the adults (F_{wing}= 2.0381, df= 43, 64; sig. at 0.01).

Population differences in FA

Analysis was confined to adult barn owls in the five populations. Pairwise comparisons were made between the populations; the results are presented in table 5.30. FA in both wing and tarsus length differed significantly between the two wild populations, but the order was reversed; FA_{tarsus} was greatest in population 2, whereas FA_{wing} was greatest in population 1.

Among the captive populations, no significant differences in FA_{wing} occurred, but differences between populations 3 and 4, and 4 and 5 were significant for FA_{tarsus}. In comparisons between the wild and captive populations, the trends were clear; FA_{tarsus} was greater in the wild populations for all six pairwise comparisons; the differences were significant in three cases. In contrast, FA_{wing} was greatest in the captive populations, significant in five of the six pairwise comparisons.

Fig. 5.7 Testing for size dependence of FA values for tarsus length, by plotting the difference between the two sides (R-L) against the mean of the two sides (R+L) / 2. A greater spread of FA values as size increases would indicate a scaling effect; no scaling effect is present in this case

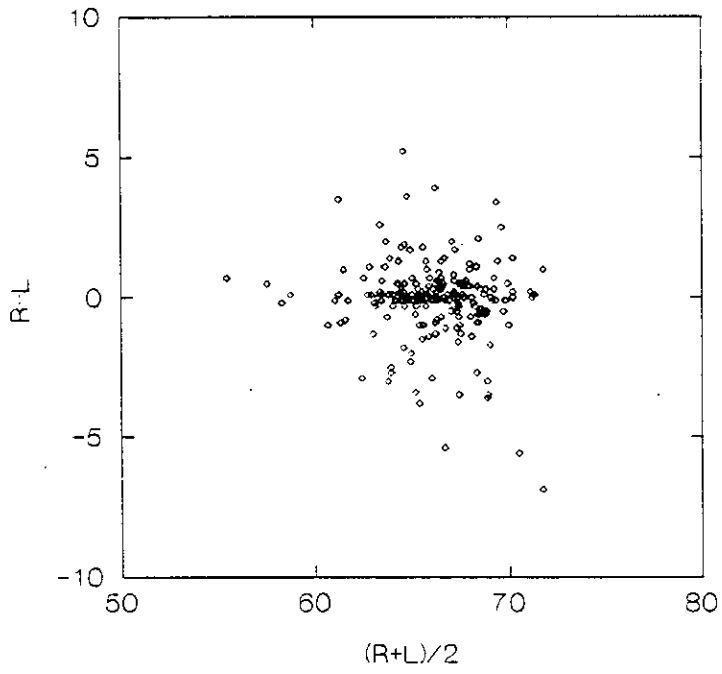


Fig. 5.8 Testing for size dependence of FA values for wing length, by plotting the difference between the two sides (R-L) against the mean of the two sides (R+L) / 2. The greater spread of FA values as size increases indicates a scaling effect

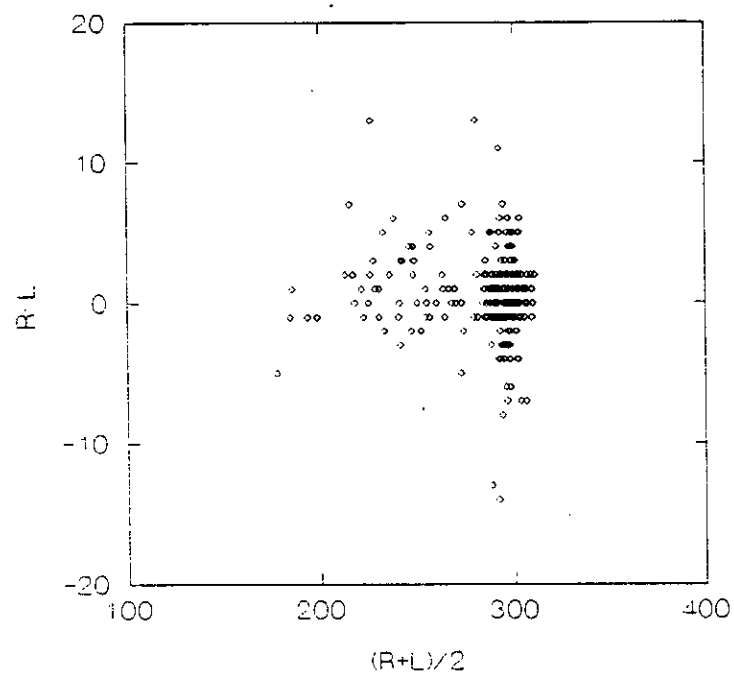


Fig. 5.9 The distribution of FA values for tarsus length, including data from both sexes, adults and juveniles, from the five study populations combined. A normal distribution curve is included for comparison.

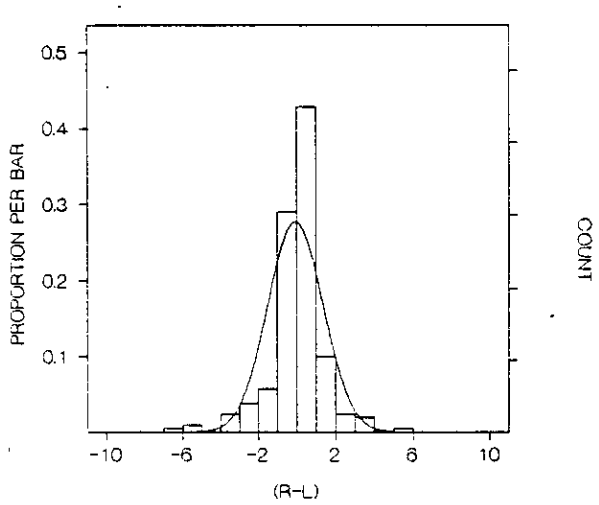


Fig. 5.10 The distribution of FA values for wing length, including data from both sexes, adults and juveniles, from the five study populations combined. A normal distribution curve is included for comparison.

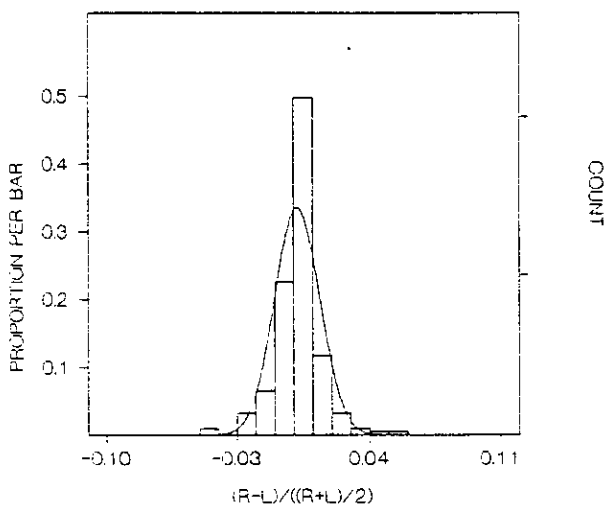


Table 5.29 FA index values for tarsus and wing lengths of adult barn owls in two wild (1-2) and three captive (3-5) populations, and for separate sex and age-classes in the largest population. n = sample size, see text for a description of the FA indices.

sub-set of data	n	FA_{tarsus}	n	FA_{wing} (* 10^{-4})
adult males, population 1	23	1.2517	24	1.0235
adult females, population 1	38	2.0180	38	0.7694
juveniles, population 1	43	1.5339	44	1.7469
adults, population 1	63	1.9554	65	0.8571
adults, population 2	45	3.8438	47	0.5210
adults, population 3	15	0.1564	14	3.7109
adults, population 4	18	1.8846	17	2.7744
adults, population 5	8	0.2955	8	1.3352

Table 5.30 F-tests to compare FA among the two wild (1-2) and three captive (3-5) populations of adult barn owls. df (2,1)=44,62 signifies 44 degrees of freedom for the population with the largest variance (2) followed by 62 degrees of freedom for the population with the smallest variance (1).

FA compared		tarsus			wing		
between populations:		F	df	sig.	F	df	sig.
wild:	1 and 2	1.9658	(2,1)=44,62	0.01	1.6452	(1,2)=64,46	0.05
captive:	3 and 4	12.051	(4,3)=17,14	0.01	1.3376	(3,4)=13,16	ns
	3 and 5	1.8898	(5,3)= 7,14	ns	2.7793	(3,5)=13, 7	ns
	4 and 5	6.3768	(4,5)=17, 7	0.01	2.0779	(4,5)=16, 7	ns
wild /	1 and 3	0.0800	(1,3)= 62,14	ns	4.3294	(3,1)= 13,64	0.01
captive:	1 and 4	1.0376	(1,4)= 62,17	ns	3.2368	(4,1)= 16,64	0.01
	1 and 5	5.2570	(1,5)= 62, 7	0.05	1.5577	(5,1)= 7,64	ns
	2 and 3	24.579	(2,3)= 44,14	0.01	7.1226	(3,2)= 13,46	0.01
	2 and 4	2.0396	(2,4)= 44,17	ns	5.3250	(4,2)= 16,46	0.01
	2 and 5	13.006	(2,5)= 44 7	0.01	2.5627	(5,2)= 7,46	0.05

5.3:4 Discussion

(a) Age differences

Young birds were measured at an age when their tarsi were fully developed, yet wing extension may not have been fully completed (chapter 3). The greater FA in young barn owl wing length may therefore be explained by slightly different rates of wing extension occurring during the measurement period; it may be expected that this asymmetry would be reduced as the wings attained adult size. Asymmetry during growth could be accentuated if the young were subject to nutritional deficiencies during the period of feather extension; evidence of fault bars on the feathers would be informative in testing this hypothesis (P. Jones, pers comm.). Even in the absence of nutritional stress, however, asymmetry would be expected to be higher in juveniles as a natural effect of growth under polygenic control (Atchley, 1984). An alternative explanation would be that young sampled in the nest are sampled pre-selection, and that the individuals with high FA are selected against before reaching breeding age. Although this can not be entirely discounted, the lack of a difference between the age groups for FA in tarsus length is suggestive that once adult body size is attained, the higher asymmetry during growth has largely been compensated for. Measurements taken throughout the development period would be required to clarify this.

(b) Population differences

In all the population comparisons where there were significant differences between FA in both tarsus and wing length, populations with the highest FA in tarsus length exhibited the lowest FA in wing length. Two possible explanations for differences in FA in tarsus and wing length in adult barn owls are:

(i) Their response to environmental stress during development varies due to their different developmental times; they have different 'windows of susceptibility' during which they are vulnerable to environmental modification.

(ii)Post developmental environmental effects alter their asymmetry: although tarsus length is likely to be constant once adult size is attained, wing length may vary due to feather wear or moult.

The greater FA for tarsus length in the wild populations compared to the captive populations may be due to the captive-bred birds having a more constant developmental environment, such as an abundance of food, in accordance with Leary and Allendorf's (1989) argument, or due to greater levels in inbreeding in the wild population. Why the wild populations should differ is less clear, but extending the argument would suggest different levels of inbreeding, or a more constant developmental environment in population 1; it may be speculated that subtle

climatic or habitat differences could influence the environmental stress in these two populations.

Why, then, should the opposite trend hold for wing length, with greater FA in the captive birds? The genetic explanation is in conflict with the results above, as greater inbreeding would in this case be indicated for the captive birds. The developmental argument indicates a greater degree of stress in the captive birds, affecting wing extension but not tarsus growth. This suggests an increase in stress after tarsus development is completed, when the young birds are close to fledging; this could correspond to a time when captive birds are handled or moved from natal cages. It is not inconceivable that such a disturbance could influence growth at a critical time; Polak and Trivers (1994) describe how repeated capture of pregnant pigtailed macaques *Macaca nenestrina* lead to increased FA in their offspring, indicating that subtle environmental stress in captivity may be a significant contributor to FA. Why FA in wing length should differ between the two wild populations remains unclear; the developmental argument would suggest greater stress in the pre-fledging period for young birds in population 1.

Alternatively, the differences in FA for wing length may be explained by post developmental effects. Abrasion of primary feathers on the wire mesh of the captive birds' enclosures could result in asymmetrical feather wear; the accidental inclusion of birds regrowing primaries after moult would also result in inflated FA values, which could explain the observed population differences.

In conclusion, the observed differences in FA for wing and tarsus length could be explained by a number of different hypotheses; the level of inbreeding in the captive or wild populations, or the environmental stresses involved cannot be determined from the data presented here. It does not appear justified to assume that captivity provides a more uniform developmental environment than that of wild con-specifics, as stresses in captivity could be subtle and unquantified. This study highlights the possibility that post developmental factors may affect FA, and should therefore not be overlooked in similar studies. FA can have limited applications in conservation management if the genetic and environmental sources of variation in FA remain unquantified.

5.4 Assessing Condition

5.4:1 Introduction

Many studies have attempted to assess the condition of live birds (e.g. Smith and Bush, 1978; Wishart, 1979; Gee et al., 1981; Ormerod and Tyler, 1990; Hernandez et al., 1990; Bolton et al., 1991; Bortolotti and Iko, 1992; Ferrer, 1992; Senar et al., 1992; Fox et al., 1992; Hamer and Furness, 1993; Dufour et al., 1993; van der Meer and Piersma, 1994). Definitions of condition vary, but the broad aim is to quantify some quality of the bird which is directly related to its survival or reproductive potential. If such an assessment could be carried out accurately, this would be a valuable tool for population monitoring or management. At the population level, seasonal changes in condition could highlight periods when the population would be most vulnerable to additional stress; differences among populations correlating with environmental variables could suggest differences in habitat quality. At the individual level, birds in poor condition could be targeted for supplementary feeding or veterinary care if the population was intensively managed for conservation; alternatively changes in an individual's condition could help explain observed patterns of mortality.

Ornithological field workers have generally assessed condition in terms of body reserves and stores (van der Meer and Piersma, 1994), and so much research focusses on devising a reliable method for estimating body condition from data easily obtainable in the field. Approaches include estimating fat or protein deposits from total body weight corrected for structural size (Wishart, 1979; Sibly et al., 1987; Piersma, 1984; Ormerod and Tyler, 1990; van der Meer and Piersma, 1994); quantifying the pectoral muscle profile (Sibly et al., 1987; Bolton et al., 1991; Bolton et al., 1993), measuring pectoral muscle thickness using ultrasound (Newton, 1993), or estimating fat or protein scores (Gosler, cited in Ormerod and Tyler, 1990).

A quite different approach to assessing a birds condition is commonly taken by veterinarians of wild or zoo populations (Cooper, 1978); in this case, blood parameters are assessed in a preliminary clinical or physiological evaluation, condition being defined in terms of trauma, injury or pathology.

These two approaches to describing 'condition' provide different kinds of information concerning the fitness of the birds, and may be complementary in an assessment of the condition of the individuals in a wild population. As examples of each approach, 'condition' in this study is estimated by body weight corrected for structural size, and by the percentage of

red blood cells in a blood sample. These methods were selected to utilise data that were collected during the course of a heritability study of morphometric traits, and blood samples that were collected for isozyme analysis.

5.4:2 Assessing body condition using weight data

Introduction

Body mass is a compound measure, including the mass of skeletal structure, organs, tissue, water content and nutrient stores. Van der Meer and Piersma (1994) define stores as "the nutrients that are accumulated in anticipation of periods of shortage", and note that although stores commonly consist of fat, some bird species have been demonstrated to store protein. Reserves are defined as tissue which may be metabolised in extreme conditions, but which would not normally be utilised as a source of energy. The levels of both fat and protein are therefore of interest when estimating a birds body condition.

Ideally, some measurement easily obtainable from a live bird would be a good predictor of the actual amounts of fat or protein in the body (Sibly et al., 1987), and studies which combine morphological measurements with an analysis of body composition have assessed the merits of several possible indices of nutrient stores based on correcting weight data for variation in structural size (Wishart, 1979; Sibly et al., 1987; Piersma, 1984; van der Meer and Piersma, 1994). The main problem with this approach is that single linear measurements tend to be poor predictors of overall body size, when body size is determined through a multivariate analysis of a large number of skeletal components (see 5.2:1); birds vary in their body proportions, and individuals may differ according to age, sex or geographical location (McGillivray, 1985 and 1989). The best estimate of structural body size may be the first principle component of a multivariate analysis of the skeleton, but this is not feasible for a study of live birds, and a single linear trait is commonly employed to correct weight data for 'size' despite these considerations. Fox et al. (1992) and Senar et al. (1992) correct weight by wing length in studies of condition in teal and siskins *Carduelis spinus*. Rising and Somers (1989) recommend tarsus length as the best linear measurement of body size in a multivariate analysis of size variation in Savannah sparrows *Passerculus sandwichensis*; correlations between tarsus length and the first principle component in eight principle component models was considered high, ranging from 0.67-0.86 in females, and 0.78-0.89 in males. Ormerod and Tyler (1990) favoured tarsus length over wing length in an assessment of body condition in dippers *Cinclus cinclus* because tarsus length was not subject to the same sources of variation such as feather wear and moult, and was a skeletal element which remains constant once adult size is attained. Tarsus length may be further recommended as it is easy to measure in live birds, with high repeatability (e.g. this study, 5.2:2). An index of nutrient stores may therefore

be derived by correcting an individuals weight by tarsus length; dividing the cube root of weight by tarsus length is preferable to avoid combining volume and linear measures. This approach can only be reliable if structural size is the only source of variation in the weight data other than nutrient levels, but this is often not the case. The weight of breeding females, for instance, includes the increased weight of their reproductive tract, calcium deposits laid down in anticipation of egg production, and the weight of developing eggs. Juvenile birds would similarly be expected to vary in weight due to skeletal growth, organ and muscle development as well as nutrient stores. Indices of nutrient stores are therefore most likely to be accurate from corrected weight data if confined to individuals without these additional sources of weight variation, such as adult males.

An index for nutrient stores is not, in itself, a measure of the birds survival potential or reproductive potential, however; birds vary in their nutrient demands according to their particular circumstances. Starvation and death is the ultimate penalty for inadequate body stores, yet a cost would also be predicted for an excess of stores in terms of flight efficiency and energy expenditure (Freed, 1981). A bird's condition should therefore be defined in terms of the departure from the optimum level; one approach is to use the deviation from the expected mean from a regression of weight corrected by an estimator of structural size (Ormerod and Tyler, 1990). Care must then be taken in the choice of individuals used to define this 'optimum', as the inclusion of birds under different ecological conditions and pressures would weaken the validity of using the population data. In recognition of this problem, Ormerod and Tyler (1990) suggest that condition indices should be based on deviations from expected values for a given "time of year, at a given time of day, for a bird of a given age and sex, in a given type of environment" if they are to be accurate estimators of condition, and note that the initial data set to allow such sub-division would necessarily be very large. Even without such strict sub-division of the data set, however, differences in condition among populations may in some cases be interpreted in biological terms; in dippers, the birds sampled on acidic streams had significantly lower condition values than those sampled on circumneutral streams, for instance, despite all other sources of variation (Ormerod and Tyler, 1990). This approach to assessing condition would therefore seem useful, and worth attempting on other populations.

In this study, body condition in a sample of barn owls is estimated from corrected weight data, as described below.

Methods

This estimate of body condition was confined to adult males for the reasons outlined in the introduction. Adult males were sampled as described in chapter 3, and assigned a sex as described in chapter 4. Condition was calculated as the deviation from the best fit line of a regression of the cube root of weight against tarsus length. The cube root of weight was used to standardise the volume and linear measurements. Population differences in the condition index scores were tested by ANOVA, nesting the two wild and three captive populations within a captive/wild category. Possible seasonal effects in wild adult males were then investigated for condition values recalculated from a regression of the sub-set of wild adult males, testing for differences by ANOVA, as before.

Results

The regression of the cube root of weight against tarsus length for 70 adult male barn owls from the five populations is shown in fig. 5.11. The deviation of the independent variable from the best fit line is taken as the estimate of condition; the distribution of condition index values derived in this way is shown in fig. 5.12. Condition index values approximate a normal distribution about a mean of 0.00, with a range from -0.05 to +0.07. The condition data are then described by mean and standard deviation for each of the five populations sampled (Table 5.31); sample size for adult males ranged from 4 from population 5 to 26 from population 1; population means for condition index values deviated from the overall mean of 0.00 from -0.087 in population 2, to +0.155 in population 3. The significance of the population differences was tested by ANOVA (Table 5.32); WILD was shown to be a significant explanatory variable for condition, with the condition index score of captive adult males exceeding that of wild adult males by 0.020. The differences among populations are shown by pairwise comparisons in table 5.33; significant differences occured between populations 1+3 and between 2+3; no significant population differences were shown within the wild or captive categories.

To test for seasonal effects in the wild barn owls, data from populations 1 and 2 were combined and the condition index recalculated from a regression as before, on this sub-set of data (n = 47). In this case, cube root of weight = 5.440 + 0.020 tarsus; r = 0.232, t = 1.633 (ns). The month in which these males were sampled is shown in table 5.34, with the mean and standard deviation of the recalculated condition index shown per month from April to August. This is represented graphically in fig. 5.13; differences in mean condition index are small, and considerable overlap of the error bars occurs. As would be expected from this graph, the ANOVA in table 5.35 shows no significant effect of the month sampled for these data.

Fig. 5.11 Regression of the cube root of weight against tarsus length for adult males from all populations: cube root of weight = 5.999 + 0.012 * tarsus. n = 70, r = 0.146, t = 1.221 (ns). The deviation of the independent variable from the best fit line is taken as the measure of condition for adult males.

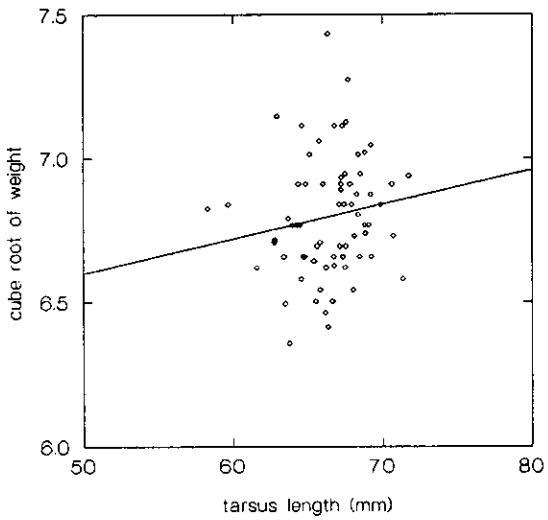


Fig. 5.12 Distribution of condition index values (residuals) for adult males; condition is defined as the deviation of the independent variable from the best fit line of the regression of cube root weight against tarsus length, as shown in fig. 5.11. n = 70.

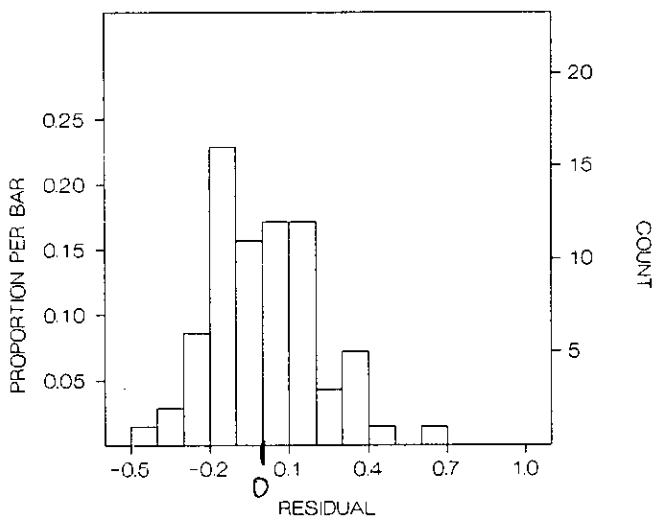


Table 5.31 Condition data for 70 adult male barn owls derived as the deviation from the relationship between cube root of weight and tarsus length, as shown in fig. 5.11. n, mean and standard deviation per population.

POPN	n	mean	S.D.
P1	26	-0.028	0.201
P2	21	-0.087	0.158
P3	13	0.155	0.221
P4	6	0.131	0.136
P5	4	-0.063	0.173
TOTAL	70	0.000	0.204

Table 5.32 ANOVA to test for population differences in condition index among adult males. Population is nested within the captive/wild WILD category.

DEPENDENT VARIABLE: ADULT MALE RESIDUALS N:70 MULTIPLE R: 0.492 SQUARED MULTIPLE R: 0.242

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
POPN {WILD}	0.230	4	0.058	1.686	0.164
WILD	0.153	1	0.153	4.480	0.038* C>W
ERROR	2.186	64	0.034		

Table 5.33 Pairwise mean differences in condition index score among populations. Bonferroni adjusted probabilities in parentheses.

		WILD			CAPTIVE	
		P1 (n = 26)	P2 (n = 21)	P3 (n = 21)	P4 (n = 6)	P5 (n = 4)
WILD	P1	0.000 (1.000)				
	P2	-0.060 (1.000)	0.000 (1.000)			
CAPTIVE	P3	0.218 (0.026) *	0.277 (0.002) **	0.000 (1.000)		
	P4	0.159 (0.939)	0.218 (0.196)	-0.059 (1.000)	0.000 (1.000)	
	P5	-0.035 (1.000)	0.024 (1.000)	-0.253 (0.332)	-0.194 (1.000)	0.000 (1.000)

Table 5.34 Means and standard deviations per month sampled, for the condition index values based on wild adult males.

MONTH	n	mean	S.D.
April	17	-0.038	0.161
May	14	0.048	0.154
June	10	0.012	0.251
July	3	-0.038	0.211
August	3	-0.004	0.210
TOTAL	47	0.000	0.179

Fig. 5.13 Condition index for wild adult males for each month sampled from April to August, to look for possible patterns in condition index throughout the breeding season in wild adult males. Populations 1 and 2 combined. n = 47; bars show ± S.E. of mean.

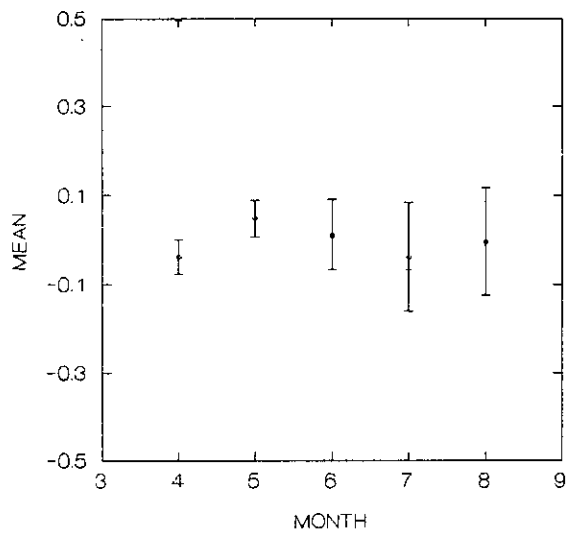


Table 5.35 ANOVA to test for differences in condition index score among the months sampled for wild adult males.

DEP VAR: WILD ADULT MALES	N: 47	MULTIPLE R: 0.200	SQUARED MULTIPLE R: 0.040	
SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
MONTH	0.062	4	0.015	0.440	0.779 ns
ERROR	1.469	42	0.035		

Discussion

There are three main sources of error in this method of assessing condition:

(1) A linear trait is used to correct the weight data for variation in structural size, despite much evidence that this is in many cases inadequate due to proportional differences among birds.

(2) It is assumed that once variation in structural size has been standardised, the remaining variation in weight data is due to the birds stored nutrients. This is unlikely to be the case in breeding females or juveniles, and these are excluded from analysis for this reason. Additional sources of variation may also be expected in adult males, however; these include water content and ingested food, which would contribute to diurnal variation in weight.

(3) The value of condition indices in predicting an individual's survival potential depends on the identification of the optimum state, but this is not easily determined in a trait assumed to be under balancing selection, when the sampled individuals are suspected to be subject to different ecological conditions and pressures. Only where data sets are large, allowing condition indices to be established for a particular age, sex , season etc. is the deviation from the population mean likely to be a good estimator of the deviation from the optimum state.

In this study, the index based on adult males showed differences among wild and captive populations, with captive males exceeding wild males in corrected weight. If the assumptions of points 1 and 2 described above are met, this result would indicate differences in nutrient stores between the wild and captive populations. Interpreting this result in terms of survival or fitness is not straightforward, however, because of the problem outlined in point 3; the defining model was based on birds in quite different environments, and the optimum state in terms of nutrient stores may well differ among the populations. The wild adult males, for instance, were sampled during the breeding season, when flight efficiency for foraging may be an important determining factor of optimum stores. The captive birds, in contrast, were sampled in winter, and efficient flight capability would not be an issue for aviary kept birds.

As described by Ormerod and Tyler (1990), this method of assessing condition may be of most use if confined to particular sub-sets of the population. In addition, due to assumptions made in the methodology, most confidence could be placed on the results if the relationship between the condition index and actual nutrient stores was established in a study of carcass composition and external measurements for the species under study.

5.4:3 Using Hematocrit to Assess Condition in Birds

Introduction

Blood parameters may be useful indicators of an individuals health, as organ malfunctions, infectious disease and trauma cause often predictable changes in blood values (van der Heyden, 1980). Of a range of available tests including cell counts and protein assays, the packed cell volume (PCV) is often favoured in preliminary diagnosis as it is quick and easy to measure, and has low measurement error in comparison to other hematological tests (Cooper, 1978; Hernandez et al., 1990; Gee et al., 1981). Smith and Bush (1978) suggest that a PCV of 10-15% below normal in birds is diagnostic of anemia due to parasites, blood loss or malnutrition, whereas an elevated PCV indicates dehydration.Wound infection, bone infection, TB/aspergillosis, viral hepatitis, lead poisoning and septicemia also correlated with lowered PCV in a study of injured and healthy owls of several species maintained in a rehabilitation centre (van der Heyden, 1980). Richner et al. (1993) demonstrated that a lowered PCV correlated with a parasite infection in a study of hen flea infestation of great tit *Parus major* nests, whereas Arad et al. (1989) induced a 12.5% increase in PCV in heat exposed dehydrated pigeons *Columbia liva*. A departure from a normal PCV may therefore be a useful, though not specific, indication of health problems in an individual.

Meaningful interpretation of PCV values is dependent on the availability of appropriate reference values; in the past, values from domestic fowl have been relied upon for the clinical evaluation of wild birds, yet Gee et al. (1981) demonstrated significant interspecific differences in PCV values for healthy individuals in a study of 12 avian species. In recognition of the need for appropriate reference values, many recent studies have provided basic hematological data for a range of bird species (e.g. Chilean flamingo *Phoenicopterus ruber chilensis*, Puerta et al., 1989; Common cranes *Grus grus*, Puerta et al., 1990; Dark eyed junco *Junco hyemalis*, Swanson, 1990; house sparrows *Passer domesticus*, Goldstein and Zahedi, 1990; Great bustards *Otis tarda*, Jimenez et al., 1991; see also table 5.36 for a summary of data from Strigiformes and other raptors). Within the Strigiformes, Smith and Bush (1978) combined data from 14 species of owl to give an average PCV (% ±S.D.) of 41.5 ±5 for 109 individuals, and Prinzinger and Misovik (1994) combined data from 14 owl species to give an average PCV of 36.07 ±4.11. Van der Heyden (1980) published an average PCV of 40.3 ±2.3 for a sample of 6 barn owls, 5 of which were described as clinically normal, and an average PCV of 45.8 for a separate study of 10 clinically normal barn owls (Elliott et al., 1975, cited in van der Heyden, 1980). In these studies, as for the majority of avian PCV studies, reference values are based on small sample sizes, and should therefore be treated with caution if normal levels are sought for clinical diagnosis.

Interpretation of PCV values is further complicated by patterns of intra-specific variation. Although van der Heyden (1980) and Gee et al. (1981) state that most blood parameters of healthy individuals remain constant within a species, there is an increasing body of evidence showing significant intra-specific variation in PCV correlating with a range of environmental variables and natural physiologial differences among individuals. Kostelecka-Myrcha and Jaroszewicz (1993) describe variation in PCV during growth and development of nestling house martins *Delichon urbica*; PCV increased in the first 6 days after hatching, followed by a constant period, and then a further increase from 16 to 20 days after hatching; overall the PCV changed from 19.7 to 47%, and they suggest that developing young normally have a lower PCV than adult birds. This is supported by studies on flamingos *Phoenicopterus ruber*, which increased their PCV in the first few months of life (Puerta et al., 1992), and free living Great bustards *Otis tarda*, where young showed lower PCV than adults sampled at the same time (Alonso et al., 1990).

Sex differences in PCV have also been demonstrated; pre-breeding female red billed queleas *Quelea quelea* had higher PCV (56.3%) than males (PCV 53.9%) (Jones, 1983), as did female Chilean flamingos *Phoenicopterus ruber chilensis* (Puerta et al., 1989). PCV in breeding female red-billed queleas was lowest on the day their first egg was laid, however; Jones (1983) suggests that this is due to nutrients being diverted to egg production in preference to red cell production. Blood levels of sex steroids may also affect PCV; Cecil and Bakst (1991) report a correlation between an increase in PCV and testicular weight in turkeys from 16-22 weeks old, yet experimental testosterone implants in house sparows *Passer domesticus* did not significantly elevate PCV (Puerta et al., 1995).

Activity levels may also influence PCV; Bordel and Haase (1993) demonstrated a signficantly lower PCV in homing pigeons that had been exercised (flights of 113-620km, PCV 51.0%) in comparison to a control group of unflown birds (PCV 54.4%); they suggest that the lower PCV would improve the blood flow, and may therefore be advantageous.

PCV has also been shown to vary with season and with altitude; PCV was lower in winter (47.2%) than in summer (52.6%) in dark eyed juncos *Junco hyemalis* (Swanson, 1990), and PCV increased from the beginning to the end of summer in a sample of wild caught male house sparrows *Passer domesticus*, despite abundant provisioning of water (Puerta et al., 1995); seasonal differences were also reported in common cranes *Grus grus*, with PCV lowest in early autumn (Abelenda et al., 1993). An altitude effect on PCV has been demonstrated

with coot *Fulica americana peruviana* embryos in Peru; PCV was significantly higher in embryos sampled at higher altitudes, compared to lowland coots (Carey et al., 1993).

This evidence suggests that if PCV is to be of use in clinical diagnosis, reference values should be computed specifically for particular age and sex classes in a population, and that other factors such as season and stage of reproductive cycle should also be taken into account. This would require a far greater data base of hematological data than is available for the majority of avian species, and would often not be feasible; the value of PCV as a clinical diagnostic tool must therefore be questionable for many species. In this study, it is aimed to provide a greater sample of PCV data for barn owls than has previously been published, and to investigate possible sex, age and population differences in PCV.

Methods

Capture and blood sampling of barn owls for PCV assessment is described in chapter 3; birds were assigned a sex as described in chapter 4. Blood samples for PCV were only taken from individuals for whom a full $1mm^3$ blood sample was obtained, as priority was placed on samples for isozyme analysis (see chapter 7). A total of 147 PCV samples were obtained from two wild (1 and 2) and two captive (3 and 4) populations.

The PCV sample was taken from the $1mm^3$ collecting tube immediately after sampling; approximately 70µl blood was drawn by capilliary action into a heparinised glass hematocrit tube, and one end was then sealed with plasticine. The samples were transported in a cool box to be processed in the laboratory, usually within eight, and always within twelve hours from sampling. Hematocrit tubes were centrifuged at 10 000g for 10 minutes, and the percentage of the red cell fraction recorded.

PCV results were analysed by ANOVA to investigate sex, age and population differences. The two wild and two captive populations were nested within a WILD category (wild =1, captive = 0); sex and age were classed as: SEX male = 1, female = 2; STAGE adult = 1, juvenile = 0.

Results

Mean PCV for barn owls combined over sex, age and population classes was 39.19% S.D. 5.198, range 25-50. This result is compared with data from other barn owl studies, owls and other raptors in table 5.36. The greatest difference between mean PCV for this barn owl study and other PCV values presented in table 5.36 occured with a sample of 2 goshawks, PCV in barn owls was 13.6% lower than the goshawk data. The PCV for this barn owl study is lower than in the two other barn owl studies, and appears low overall in comparison to the other studies. Variances are compared by F tests for those studies with a sample size greater than 3 which published n, mean and standard deviation; PVC in this study differed in variance in 6 of the 12 pairwise comparisons. Means are compared by t-tests for cases where variances did not differ significantly at the 0.05 level; this barn owl study had a significantly lower PCV than PCV in the studies on short-eared owls, Screech owls and a PCV value calculated as the mean of 14 Strigiformes, but the difference in PCV score was small, varying from 2.3 to 3.3%. No significant difference between the means was found in comparisons of this barn owl study with those of South American snail kite, Arctic peregrine falcon and common buzzard.

The distribution of PCV scores in this study is shown in fig. 5.14, and the means, S.D. and range for these birds separated by sex, age and population are shown in table 5.37. The nested analysis of variance shown in table 5.38 indicates that sex, stage and wild are significant explanatory variables for PCV. The greatest mean difference in PCV scores is shown for stage, with the adult PCV score exceeding that of juveniles by 6.52%; sexual dimorphism is weakly significant, with males exceeding females in PCV by just 1.08%. The effect of populations classed as wild or captive is highly significant, with captive barn owls exceeding wild birds in PCV by 4.69%. Pairwise comparisons of interpopulation differences (table 5.39) show that there are significant differences in PCV for all captive-wild pairwise comparisons, but no significant differences between populations within wild or captive categories. The effects of sex, stage and wild factors are further dissected by a series of pairwise comparisons, shown in table 5.40 with Tukey and Bonferroni adjusted probabilities. In these comparisons, no significant effect of sex was found when the data were sub-divided into age and captive/wild categories. Stage was a highly significant effect in wild barn owls, with adults exceeding juveniles in PCV for both sexes, but no significant difference in PCV between adults and juveniles was found in captive barn owls. Differences in PCV between captive and wild birds were significant in both adult and juvenile females, but not for males.

Table 5.36 PCV scores for barn owls, other Strigiformes, and raptors; the scores are listed from highest to lowest mean PCV in each category for ease of comparison. The F value compares variances between this barn owl study (n=147) and each other study for which n>3 and n, mean and S.D. are provided. Where variances are not significantly different at 0.05 level, means are compared with the students t-test.

Species	n	mean PCV	SD	range	source	F (p 0.05)	t
Barn owl *Tyto alba*	10	45.8			Elliott et al., 1975, cited in van der Heyden, 1980		
Barn owl *Tyto alba*	5	40.3	2.3		van der Heyden, 1980	5.112 *	
Barn owl *Tyto alba alba*	147	39.2	5.2	25-50	this study		
Short-eared owl *Asio flammeus*	6	43.5	3.7		van der Heyden, 1980	1.975 ns	1.989*
Screech owl *Otus asio*	19	42.4	4.7		van der Heyden, 1980	1.224 ns	2.524*
barred owl *Strix varia*	8	42.5	1.7		van der Heyden, 1980	9.356 *	
Great horned owl *Bubo virginianus*	29	41.5	3.1		van der Heyden, 1980	2.814 *	
Strigiformes (14 species)	109	41.5	5.0		Smith and Bush, 1978	1.082 ns	3.539**
Snowy owl *Nyctea scandiaca*	2	37.5	2.1		van der Heyden, 1980		
Strigiformes (11 species)	-	36.1	4.1	31.0-43.7	Prinzinger and Misovic, 1994		
Goshawk *Accipiter gentilis*	2	52.8	3.2		Hunter and Powers, 1980		
American kestrel (males) *Falco sparverius*	14	52.7	3.2		Hunter and Powers, 1980	2.641 *	
American kestrel (females) *Falco sparverius*	18	52.2	3.0		Hunter and Powers, 1980	3.004 *	
Red-tailed hawk *Buteo jamaicensis*	2	48.5	7.8		Hunter and Powers, 1980		
Coopers hawk *Accipiter cooperii*	1	45.0			Hunter and Powers, 1980		
South American snail kite *Rostrhamus s. sociabilis*	10	42.0	4.0	35-47	Gee et al., 1981	1.690 ns	1.648 ns
Andean condor *Vultur gryphus*	9	42.0	3.0	39-48	Gee et al., 1981	3.004 *	
Arctic peregrine falcon *Falco peregrinus tundrius*	9	42.0	4.0	35-48	Gee et al., 1981	1.690 ns	1.576 ns
Marsh hawk *Circus cyaneus*	1	41.0			Hunter and Powers, 1980		
Common buzzard *Buteo buteo*	22	40.8	4.4	49-36	Hernandez et al., 1990	1.397 ns	1.366 ns

Fig. 5.14 Histogram showing the distribution of hematocrit scores (percentage of red blood cells by volume:PCV) for a sample of 147 barn owls; sex, age and populations combined (mean = 39.19; S.D.= 5.198; range= 25-50)

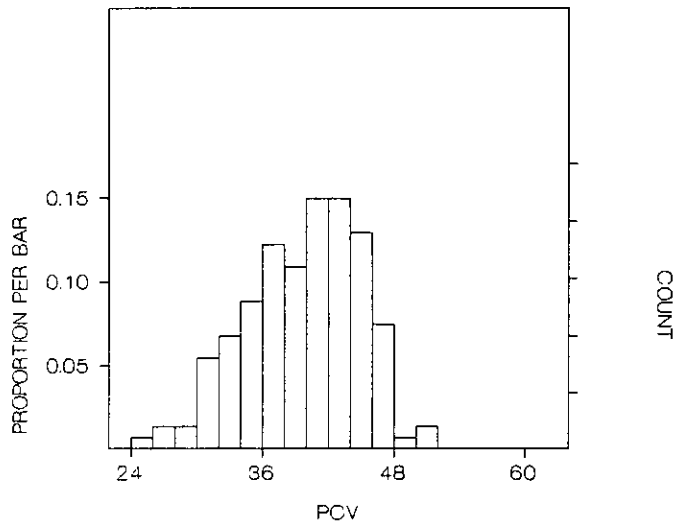


Table 5.37 Summary statistics of PCV for 147 barn owls, separated according to sex, age and population

	MALES								FEMALES							
	ADULTS				JUVENILES				ADULTS				JUVENILES			
popn	n	mean	S.D.	range	n	mean	S.D.	range	n	mean	S.D.	range	n	mean	S.D.	range
P1	23	42.61	2.692	38-47	19	35.37	3.499	30-42	28	40.43	4.977	26-50	20	33.60	3.455	26-38
P2	7	41.71	2.752	38-46	3	33.00	1.000	32-42	5	37.20	3.493	34-43	2	35.00	5.657	31-39
P3	5	42.00	4.848	37-50	3	40.67	0.577	40-41	7	44.71	2.498	40-47	1	41.00		
P4	4	44.00	1.826	42-46	1	41.00			6	42.83	3.764	38-48	1	45.00		
total	39	42.51	2.919	37-50	26	35.92	3.730	30-42	46	41.04	4.770	26-50	26	34.50	4.314	26-45

Table 5.38 Analysis of variance to test for differences in PCV scores among sex, age and population groups. Populations 1-4 are nested within the wild category (1=wild, 0=captive).

DEP VAR: PCV N: 135 MULTIPLE R: 0.693 SQUARED MULTIPLE R: 0.481

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P	PVC SCORE: MEAN DIFF.
POPN{WILD}	27.344	2	13.672	0.958	0.386	
WILD	290.916	1	290.916	20.391	0.000***	C>W by 4.69
SEX	68.760	1	68.760	4.819	0.030*	M>F by 1.08
STAGE	1149.579	1	1149.579	0.575	0.000***	A>J by 6.52
SEX*STAGE	1.994	1	1.994	0.140	0.709	
ERROR	1826.195	128	14.267			

Table 5.39 Matrix of pairwise mean differences among populations (nested within 'wild' category) for PCV score, as computed in the above ANOVA; Bonferroni adjusted pairwise comparison probabilities in parentheses. P1 and P2 are wild, P3 and P4 captive populations.

		CAPTIVE		WILD	
		P3 (n = 16)	P4 (n = 12)	P1(n = 90)	P2 (n = 17)
CAPTIVE	P3	0.000 (1.000)			
	P4	-0.026 (1.000)	0.000 (1.000)		
WILD	P1	-3.320 (0.010**)	-3.294 (0.035*)	0.000 (1.000)	
	P2	-4.714 (0.003**)	-4.688 (0.008**)	-1.394 (1.000)	0.000 (1.000)

Table 5.40 Paired comparisons in PCV among sex, adult/juvenile and captive/wild categories. Pairs are tested with Tukey's probabilities and Bonferroni adjusted probabilities for comparison- adjusting the probabilities for multiple tests did not result in different assigning of significance levels in these comparisons.

Factor under test	Paired comparison (n)	Mean difference in PCV	Tukey	Bonferroni
SEX	wild adult males (30) wild adult females (33)	2.461 males>females	0.136	0.249
SEX	captive adult males (9) captive adult females (13)	0.957 females>males	0.999	1.000
SEX	wild juvenile males (22) wild juvenile females (22)	1.318 males>females	0.935	1.000
SEX	captive juvenile males (4) captive juvenile females (2)	2.250 females>males	0.997	1.000
STAGE	wild adult males (30) wild juvenile males (22)	7.355 adult>juvenile	0.000***	0.000***
STAGE	wild adult females (33) wild juvenile females (22)	6.212 adult>juvenile	0.000***	0.000***
STAGE	captive adult males (9) captive juvenile males (4)	2.139 adult>juvenile	0.979	1.000
STAGE	captive adult females (13) captive juvenile females (2)	0.846 adult>juvenile	1.000	1.000
WILD	wild adult males (30) captive adult males (9)	0.489 captive>wild	1.000	1.000
WILD	wild adult females (33) captive adult females (13)	3.907 captive>wild	0.026*	0.041*
WILD	wild juvenile males (22) captive juvenile males (4)	5.705 captive>wild	0.081	0.139
WILD	wild juvenile females (22) captive juvenile females (2)	9.273 captive>wild	0.015*	0.024*

Discussion

Patterns of PCV variation

The differences among mean PCV scores for different species summarised in table 5.36 appear initially to suggest interspecific variation in PCV among owls and raptors, in accordance with the interspecific comparisons for other birds described by Gee et al. (1981). These comparisons, however, are based on small samples of birds of unknown sex, age, season of sampling, activity levels etc., and the differences are often small. As these differences in PCV scores are within the limits of variation described in intraspecific studies attributable to age, sex or a range of environmental influences (see section 5.4:3, Introduction), conclusions concerning interspecific differences in PCV are not justified on the basis of these data. Interspecific PCV variation would only be convincingly demonstrated if intraspecific variation was quantified and explained for each species, allowing more meaningful comparison of variation within sex, age or other sub-groups of the data.

The highly significant and relatively large difference in PCV between adult and juvenile barn owls in this study is in accordance with the trend described by Kostelecka-Myrcha and Jaroszewicz (1993), Puerta et al. (1992) and Alonso et al. (1990) of a higher PCV in adult than in juvenile birds. Kostelecka-Myrcha and Jaroszewiczs (1993) study of PCV throughout the growth period of nestling house martins showed an increase in PCV as development progressed; as the juvenile barn owls in this study were sampled when close to fledging, it is possible that age effects would be even more pronounced if the juveniles were sampled at an earlier stage.

Sex differences in PCV in this study were small, and although weakly significant overall, were not significant in pairwise comparisons of separate age and population classes. A more detailed study of PCV would be required to fully investigate possible sexual dimorphism in this trait, taking season, stage of breeding cycle and activity levels into account. The significant difference between wild and captive barn owls in this study was confined to females, and is not easily explained in terms of the possible different physiological condition between wild breeding and captive non-breeding birds, as the difference was also significant in juvenile females. Given the large number of factors which correlate with PCV values shown in the literature of avian PCV values, such differences are difficult to interpret with confidence. The usefulness of these results in assessing condition is discussed below.

Using PCV to assess condition

The usefulness of PCV as a diagnostic tool in clinical evaluation depends on PCV changing in a predictable way in response to the occurence of pathogens or injury. Other sources of variation confound these effects, however; in clinically normal birds these include interspecific, sex and age differences, and a range of other factors such as activity patterns, altitude or seasonal effects; in this study, significant effects of sex, age and population were found. Where reference values are based on species means, only very extreme departures from the normal PCV values are diagnostic of health problems, due to a naturally large range of PCV values. The ±15% level suggested by Smith and Bush (1978) encompasses all the birds in this study, as ±15% from the overall PCV mean of 39% gives a clinically normal range of 24-54% compared to the actual range of 25-50%.

Where some of the sources of variation in PCV can be controlled for, it is possible that the usefulness of PCV as a diagnostic tool would be increased; reference values established for clinically normal individuals for a given species, sex and ageclass would reduce some of the natural sources of PCV variation. Less extreme departures from normal levels may then be more closely correlated with the incidence of health problems. This study provides PCV data

for adult and near fledging barn owls, but the usefulness of these reference values in assessing the birds condition could only be evaluated by correlating deviations from normal PCV scores with some independent assessment of condition such as the incidence of pathogens, parasite load, or known injury.

Conclusions on assessing condition

The two approaches to assessing condition described here deal with quite different aspects of a birds survival potential. Condition defined in terms of body stores may be the most relevant in a wild population study, as differences in body stores affect many parameters of interest, such as an individuals ability to survive periods of food shortage, the timing of breeding or the number of eggs produced (van der Meer and Piersma, 1994). The PCV assessment of condition, in contrast, may be of most use in identifying individuals which show an extreme deviation from the population mean, as many factors contribute to variation in clinically normal birds. As may be expected, the correlation between condition indices based on these two methods is low; in this study, PCV condition was calculated as the deviation from the mean value for wild adult males, and the correlation between this and the body weight condition index calculated for the same individuals was $r = -0.269$ (ns) $n = 29$.

Both methods require reference values if the indices are to be interpreted in any meaningful way, and in each case, an adequate reference value for the optimum state should be calculated for sub-sets of the population, as optimum values may be expected to differ among age and sex classes, and according to the season, stage of reproduction etc. For both methods of estimating condition, it would also be desirable to have some independent means of evaluating the index, by correlating the index values with data concerning carcass composition or directly correlating the index with survival or fecundity for the weight index, and with reference to clinically normal and abnormal birds and their subsequent survival in the case of PCV. In conclusion, both of the methods discussed here may provide useful information about an individuals 'condition', and as they assess different aspects of an individuals survival potential, they may be thought of as complementary in a population study.

5.5 SUMMARY: Implications for conservation management

In this chapter, three approaches to analysing quantitative data, relevant to population management, were taken.

The first approach was to examine three quantitative traits easily measured on live birds for their potential for heritability analysis. This is relevant to population management if the population is to be monitored in the long term for possible loss of genetic variability in metric traits, as the observed phenotypic variance should be interpreted in terms of its genetic and environmental components if predictions about the potential response to selection are to be made. This preliminary analysis enabled wing length to be rejected in this study, but tarsus length and weight were considered suitable for further analysis. The heritability of these traits is discussed in the following chapter.

The second approach involved calculating the departure from bilateral symmetry for tarsus and wing length; departures from bilateral symmetry have been described as the best available method of determining phenotypic quality, as the optimum state (perfect symmetry) is known. An increase in FA denotes developmental instability, but the population management implications of such a study are not clear if the underlying cause of the imbalance is not known. An increase in asymmetry may be due to an increase in environmental stress, or to a decrease in the individuals ability to compensate for environmental stress, as occurs in inbred individuals. In this study, conflicting patterns of FA from wing and tarsus data emphasised the influence of post-developmental sources of variation in a study of FA. Without a method of assessing the underlying causes of observed patterns of FA, this approach is likely to be of limited use in wild population studies.

The third approach involved comparing two methods of assessing 'condition' in birds; one concerning body stores as estimated by weight corrected for variation in structural size, and one concerning pathological condition as assessed by a blood parameter (PCV). Both methods were hampered by the lack of relevant reference values to aid interpretation of the data, and the value of either approach depends on establishing 'optimum' values for comparison. In both cases, large data sets, allowing sub-division into relevant ecological units is desirable. Although a method of assessing nutrient stores is more widely useful than identifying birds which depart markedly from being clinically normal in a wild population study, both methods may be complementary in explaining observed patterns of mortality, or identifying individuals

in an intensively managed population which would benefit from supplementary feeding or veterinary care.

In many of the analyses in this chapter, the interpretation of observed patterns of phenotypic variation is limited because of the unknown contribution of genetic and environmental sources of variation. The problem of partitioning phenotypic variance into genetic and environmental components is addressed in the following chapter.

Chapter 6

HERITABILITY OF QUANTITATIVE TRAITS

6.1: Introduction

Interpretation of the observed patterns of variance described in chapter 5 was often limited because the underlying causes of variation in the metric traits remained unquantified. Measurement error (5.2:2) is a purely environmental component of variance, yet for all the other topics covered- intercorrelations among traits (5.2:1), population differences (5.2:3), sexual dimorphism (5.2:3) and fluctuating asymmetry (5.3), the same question arises: to what extent was the observed variance due to differences in genotype or to environmental factors?

Partitioning observed phenotypic variation in quantitative traits into its constituent genetic and environmental parts has long been of interest to animal breeders and evolutionary biologists. If the proportion of the variability in a given trait which is due to the additive effects of genes can be estimated, predictions can be made about the organism's response to selection on that character. This proportion is termed the heritability, and is described by Falconer (1981, Ch. 10) as one of the most important properties of a metric trait.

Heritability is clearly a very important parameter for the conservation biologist, who may wish to monitor a wild population and predict its response to environmental change, or to predict the likelihood of genetic change occurring when a species is bred in captivity. Methods which are routinely used on animals of commercial interest or in laboratory experiments to estimate heritability have also been applied to natural populations (avian examples reviewed in Boag and van Noordwijk, 1987). In this chapter these methods are applied to data collected from a wild population of barn owls, to estimate the proportion of variance which may be attributable to the additive effects of genes for the metric traits discussed in chapter 5.

6.1:1 Heritability: definition

Heritability is defined as the ratio of additive genetic variance to phenotypic variance:

$$h^2 = V_A / V_P$$

(Falconer, 1981, Ch. 10)

Its estimation depends on measuring traits on individuals of known relatedness, as relatives will tend to resemble each other phenotypically because of the genes they share.

The observed phenotypic variance in the heritability estimate consists of the additive genetic variance plus environmental, dominance and interaction deviations.

- Dominance deviations occur in full siblings, which may resemble each other more closely than they resemble either parent, if due to recombination they share pairs of alleles which were not paired in their parents, and whose expression is phenotypically different to their parents' alleles at that locus.

- Interaction (epistatic) deviations describe the departure from the additive combination of genotype values which occurs when a genotype is the result of two or more interacting loci.

- The environmental deviation describes the effect of any aspect of the environment which influences the phenotype, such as food quality and quantity, or fluctuating temperatures.

It follows from the above definition that a heritability estimate is specific to the trait and population for which it was determined, and to the environment in which it was measured. It can not be extrapolated to other populations, as they are likely to differ with respect to both their genetic composition and to their environment; nor should data from separate populations be combined. The effect of combining data from distinct populations will depend on the relative genetic and environmental differences between them; heritability is more likely to be lowered due to increased environmental variance.

6.1:2 Range of heritability values

A heritability value of 1 indicates a complete correlation between parents and offspring; parental values for the phenotype predict the offspring values. Boag and van Noordwijk (1987) review heritabilities in a range of traits for wild bird populations; most external morphological characters had heritabilities in the range of 0.60-0.70, whereas traits closely involved with reproductive fitness had lower heritabilities of 0.30-0.40.

This is consistent with Falconer's review (1981) of heritability estimates on various animal species, in which morphological characters had heritabilities of 0.35 (body weight in mice) to 0.70 (back-fat thickness in pigs); for traits important for reproductive fitness, the lowest heritability was 0.05 for litter size in pigs, ranging up to 0.50 for egg weight in poultry. Exceptions exist to this generalisation, however; for example, Lessels et al.,(1989) describe egg weight heritability in lesser snow geese *Anser caerulescens caerulescens* at 0.60, whereas clutch size heritability was 0.15.

The range of heritability estimates is not surprising, when it is remembered that the heritability value represents a ratio, and is therefore affected by any factors which influence the components of variation described above. Any factor which increases the environmental component of variance tends to reduce the heritability, as does any factor which reduces the genetic component. Selection over generations for traits important in reproductive fitness will have reduced the amount of genetic variability present, and so observed variability in this case is largely environmental in origin. Small populations in which fixation of alleles had taken place due to drift or inbreeding would similarly be expected to have low heritability values.

The highest heritabilities are seen in characters which have either not been narrowly constrained by past selection, or are little influenced by the environment. This may be due to constant environmental conditions for the particular trait, such as unrestricted diet in the case of weight or fat measurements, or to characters whose variation has only minor influence on the fitness of the animal, such as abdominal bristle number in *Drosophila.*

Houle (1992) describes how simply comparing heritability estimates among traits does not allow their relative responses to selection to be determined, as the heritability ratio does not give information on the actual amount of phenotypic variation upon which selection acts. Houle recommends the use of trait means to standardise genetic variances for comparative purposes; data from 200 studies of animal quantitative genetics reviewed by Houle, and standardised in this way, showed traits that were closely related to fitness to be more variable than morphological traits, in contrast to the conclusions often drawn from heritability studies alone. Houle therefore stresses the value of including data on trait means and variances as well as heritability estimates in studies of quantitative genetics.

6.1:3 Methods available for estimating heritability

Some studies have estimated heritability by manipulating levels of selection, and inferring heritability from the magnitude of the population's response. This type of analysis has a limited practical use for wild populations, however, as it can not be assumed that selection has acted on the trait of interest to produce the observed response (Boag and van Noordwijk, 1987).

Heritability is usually estimated from the similarity between relatives for a given trait, as described by Falconer (1981). As offspring receive half their genes from each parent (ignoring the sex chromosomes) the mean of the offspring values in a family would equal the mid-parent value, if all their observed phenotypic variation was due to the additive effects of genes. The

greater the environmental component of the phenotypic variation, the closer the offspring resemble the population rather than the parental mean.

Commonly regressions of offspring on parents (single sex or mean values) comparing phenotype between generations, or analyses of variance between siblings, comparing phenotype within generations, are used to estimate heritability.

Although simple to execute, parent-offspring regressions and sib analyses are limited to particular relationships and therefore may not make optimal use of the available data. Where data are unbalanced and pedigrees complicated, maximum likelihood techniques may be favoured (Shaw, 1987), as all pedigree data are included in the analysis. Dohm and Garland (1993), for instance, used REML to estimate heritabilities in garter snakes *Thamnophis sirtalis*, where litter size ranged from 1-26 offspring; Price and Burley (1993) used REML to estimate heritability of zebra finch *Taeniopygia guttata* bill colour where pedigree was too complicated to allow for the standard calculations. REML has the additional advantages that it is robust to departures from normally distributed data, and tests for the significance of genetic and environmental correlations are possible; it would therefore be particularly useful for heritability estimates in natural populations (Shaw, 1987).

Despite the potential benefits of a maximum likelihood approach to heritability, regressions and analysis of variance persist as the most commonly used techniques for heritability estimates in natural populations, due to their relative ease in calculation. If these methods are to give good estimates of heritability, however, certain assumptions must be met. The data should be normally distributed, and outliers also have a large influence on heritability estimates. Assumptions include an absence of correlated environments, genotype-environment correlations and genotype-environment interactions (Findlay and Cooke, 1983); fertility and viability must not be correlated with the phenotypic value of the character (no selection) (Falconer, 1981, Ch. 10), and there should be no assortative mating (not a problem for offspring- mid parent regressions). (Boag and van Noordwijk, 1987). Where these criteria are not met, large biases may be introduced in the estimation of heritability.

It may be inferred from the above account that heritability estimates calculated for a particular trait in a population are likely to differ according to the method of estimation chosen; each makes different use of pedigree data, and so the estimates are likely to differ both in terms of the amount of bias and the degree of precision. A method for estimating heritability may be chosen according to the relative importance attached to these factors; precision and bias in heritability estimates for natural populations are discussed below.

6.1:4 Precision of heritability estimates

Heritability estimates are most precise when derived from large samples of close relatives (Falconer, 1981). In studies in which the breeding stock can be manipulated, this presents no problem, as large samples of close relatives have only financial and temporal limits. Aggrey and Cheng (1992), for example, calculated heritability of body weight in a sample of 702 young Silver King X White King pigeons, from 144 pairs; 13801 chickens over four generations were used in Singh et al.s' estimates (1990) for heritability of body weight in broilers.

In natural populations the relatives available for study are determined by their usual population constraints, for example food availability influencing brood or litter size. The population may in any case be small by experimental standards (10s rather than 100s of breeding adults). Heritability estimates for wild populations might therefore be expected to be less precise than similar studies under controlled breeding. Standard errors for heritabilities of various traits in man, cattle, pigs, poultry, mice and *Drosophila*, reviewed in Falconer (1981), are in the range of 2 to 10%. Boag and van Noordwijk (1987) describe large standard errors for avian heritability estimates on wild populations when population size is small, yet significant results have been published for a range of characters in wild birds. Alatalo and Lundberg (1986), for example, give a heritability for tarsus length in a population of pied flycatchers *Ficedula hypoleuca* in Sweden of 0.53 ∓0.10, from mother-offspring regressions. Tarsus length in blue tits *Parus caeruleus* had a heritability of 0.61 ∓0.13 for mid-parent/mean offspring regressions (Dhondt, 1982). Clutch size in a population of lesser snow geese *Anser caerulescens caerulescens* had a heritability of 0.61 ∓0.19 from mother-daughter regressions (Findlay and Cooke, 1983). The common factor in these studies is a large sample size, and published data on heritability in wild populations is probably biased towards species for which large populations of easy to sample parents and offspring are available.

6.1:5 Bias in heritability

Environmental factors which result in relatives resembling each other tend to inflate heritability estimates. Even in experiments where environmental conditions can be controlled, offspring may resemble their mother more closely than their father for traits related to body size. This type of genotype-environment correlation, the maternal effect, occurs in birds when a mother who is genetically large passes not only the genetic potential to be large to her offspring, but a larger share of resources, due to the size or quality of the egg she lays. Genotype-environment interactions may exaggerate this effect; in natural populations, where the environment is typically patchy with respect to resources, large parents will occupy the

better breeding sites if their size gives them a competitive advantage. They are then better able to invest resources in their offspring. In this way, male parents are also involved in inflated heritability estimates.

The situation is more extreme for full siblings; their resemblance due to a common nest environment may be independent of their resemblance to either parent; their parents are likely to have developed at a different nest site and in a different year, yet the siblings share these conditions. As stated in the definition of heritability, dominance interactions further inflate heritability estimates in the case of siblings.

For these reasons, heritabilities based on full sibling analyses are the most likely to be inflated, and such results can usually only set an upper limit to heritability. Offspring-mother regressions are more likely to be biased than offspring-father regressions. Falconer (1981) highlights heritability estimates from half-sibling correlations and offspring-father regressions as the estimates least likely to be influenced by these factors.

Common environment effects can be investigated or minimised in controlled populations, easily carried out in the laboratory by randomising the genotypes across environments. In natural populations of birds, this has been attempted by cross fostering experiments. Dhondt (1982) for example, swapped clutches of blue tits *Parus caeruleus* and compared the heritabilities of tarsus length for normal and cross-fostered broods. The heritability estimates based on mid-parent/mid offspring regressions were similar for offspring reared by their real parents (0.61 ∓0.13), and for offspring regressed on their real parents but reared by foster parents (0.64 ∓0.18), indicating that post hatching environmental effects on parent-offspring resemblance were not significant. Maternal effects can be investigated by comparing male parent-offspring and female parent-offspring heritabilities, if paternity is assured; in the case of the blue tits, male-offspring heritability for tarsus length was 0.61 ∓0.23; female-offspring heritability was 0.68 ∓0.18, indicating that maternal effect through egg size or quality was low or non-existent. Similar results were obtained for song sparrows *Melospiza melodia* (Smith and Dhondt, 1980); Dhondt concluded that heritabilities calculated for other bird populations would be similarly free from common environment or maternal effects. Boag and van Noordwijk (1987) suggested that maternal effects fade fairly quickly during development in natural populations of birds.

In Alatalo and Lundbergs' study of pied flycatchers (1986), although cross fostering revealed no influence of the nest environment on the parent-offspring resemblance, shared environment effects were demonstrated. In this case, heritability estimates for tarsus length based on full

siblings were double the values for the offspring-parent regressions (1.148 for full sibs, 0.053 ∓0.10 for offspring-mother). Inflated heritability values obtained from full-sibs are due to dominance effects or shared environment effects. Different levels of food provisioning were inferred in this population, with reductions in tarsus growth occurring with polygyny, high breeding density and lateness of the breeding season. The discrepancy in the heritability estimates may therefore be due to offspring in a nest resembling each other more closely than they resembled offspring in nests which were subject to different levels of feeding; the common nest environment inflated the full-sib heritability estimates in this study.

Schluter and Gustafsson (1993) further investigated the possibility of maternal effects in wild populations. Their aim was to find a quantitative estimate for maternal effect on the heritability of clutch size in collared flycatchers *Ficedula albicollis*, using the degree of resemblance between mothers and daughters. They used the covariances among clutch size of mothers and daughters breeding in their first and second years- the genetic component is the same each year, but the condition of the breeding birds varies. In this way they demonstrated a large maternal effect on daughters' clutch size; adding one hatchling to a female's nest reduced her daughters' clutch size by 0.25 eggs. Maternal condition had a large and positive effect on daughters condition if clutch size was controlled for; heritability for clutch size was 0.33, with a large error making it borderline significant ($p = 0.06$).

These studies highlight the dangers of extrapolation; the absence of environmental interactions or maternal effects for one trait in a population really tells us nothing about the situation for other traits, populations or species. Schluter and Gustafsson's study indicates that maternal effects could strongly influence an evolutionary response to selection in birds, and should therefore not be ignored if the motivation for a heritability study is to assess the possible responses to selection. Rather than the traditional approach of selecting a method which minimises the influence of common environment or maternal effects, it would seem appropriate to employ as many complementary techniques as possible, to gain a greater understanding of the processes occurring in natural populations.

This is explored in the following section, using offspring-parent regressions and sibling analyses to estimate heritability in a population of wild barn owls.

6.2: Methods for estimating heritability in a barn owl population

6.2:1 Data collection

The data required for these analyses were metric traits measured on birds of known pedigree. Data on the metric traits weight, tarsus and wing length were collected from adult and juvenile barn owls from the five study populations, as described in chapter 3. On the basis of the analyses in 5.2, weight and tarsus length were selected as potentially suitable for heritability analysis. It was aimed to sample juvenile birds when they had attained adult size, but before they fledged, so that parental and juvenile traits would be directly comparable. Birds were assigned a sex according to their breeding records or plumage, as described in chapter 4. Pedigree data were available from ringing records for the wild birds, and from breeding records for the captive-bred barn owls. Right and left measurements of tarsus length were combined as mean values, due to the high correlation between the two sides (5.2:1).

Data from metric traits from barn owls of known pedigree were available for all five populations studied, but the sample sizes for populations 2-5 were small, consisting of 2-7 families. As it is inappropriate to combine data from different populations for heritability estimates (6.1), all estimates were confined to barn owls from the main wild population, population 1. The pedigree data for the 30 families in population 1 are shown in appendix 1; families consisted of one to seven offspring with one or both parents, or at least two siblings if no parents were sampled. Although the majority of offspring were sampled when juvenile, a small proportion were measured as adults, their parentage known from ringing records. Pedigree data were available for a total of 114 barn owls in this population.

6.2:2 Preliminary data analysis

As the data available for heritability estimates consisted of measurements from adult and juvenile birds of both sexes, the data were first examined for differences among the four classes:

- adult males
- adult females
- juvenile males
- juvenile females

as inequalities among these classes could have implications for heritability estimates (see below). Distributions were compared by imposing curves of normal distribution on histogram plots of the data; differences in variances were tested by F-tests, and where variances were similar, means were compared by analysis of variance.

6.2:3 Analysis of parent-offspring data

Heritability may be estimated from regression of parental values on offspring. If the mid-parent value is regressed on the mean offspring value, the slope of the regression is directly an estimate of heritability, and the standard error of the regression is taken as the S.E. of the estimate. If the sexes are treated separately, the regressions and their standard errors are multiplied by 2 to give the heritability (Falconer, 1981).

Falconer (1981) notes that the covariance of offspring and mid-parent values only equals the additive genetic variance if the variance is the same in both sexes. When variances differ, heritability should be estimated separately for each sex, and corrections are necessary for the mixed sex regressions.

For male offspring- female parent regressions, the regression and standard error are multiplied by:

female S.D./ male S.D.

(S.D. = standard deviation)

conversely, for the female offspring-male parent regression, the correction factor is :

male S.D / female S.D.

The unstated assumption here is that there is no difference in the variances between the parent and offspring populations; sexual dimorphism is the only source of bias in the calculations. This would normally be the case in a population not subjected to selection, if the same characters are measured in each generation. A measurement taken on a juvenile, however, may not be strictly equivalent to the character measured on an adult; variances may be unequal among generations, and this would introduce bias to heritability estimates. A correction factor of:

parent S.D. / offspring S.D.

was therefore introduced for subsequent heritability estimates; this takes into account both age and sex effects. Mid-parent values and mean offspring values were not used if the sexes differed in their variances, and the data from offspring sampled when adult and juvenile were not combined if age differences occurred.

Heritability estimates were derived from the parent/offspring data for single sex regressions, and from combined sex regressions where this was appropriate; estimates with and without the above correction factors were compared.

6.2:4 Analysis of full sibling data

Variation within and among families was first compared for young offspring sampled at their natal site, to test for significant differences among families/sites by ANOVA. As the analysis was restricted to one age class, the possible bias of unequal variances between age classes encountered in the regression analysis was avoided; where differences in mean or variance were shown between the sexes, the sexes were analysed separately.

Heritabilities were calculated from siblings by the following method:

An ANOVA is performed on the sibling data to partition the variance into within family variance σ^2_w and among family components. The expected mean square for within family variance is given by the within family mean square in the ANOVA; the expected mean square among families is:

$$\sigma^2_w + n\sigma^2_b$$

where n = number of offspring per family, and σ^2_b = among family variance.

When family size varies, n is corrected: $n = (n_1^2 + n_2^2 + n_3^2 \ldots) / N$, where N = the total number of individuals.

Hence σ^2_b = (mean square among families - mean square within families) / n

The intra-class correlation (t) is then calculated:

$$t = \sigma^2_b / \sigma^2_b + \sigma^2_w$$

(after Snedecor and Cochran, 1980)

For full-sibling families t is an estimate of half the heritability. The variance for t is:

$$\sigma^2_t = 2[1+(n-1)\,t\,]^2 (1-t)^2 / n(n-1)(N-1)$$

where n = the corrected number of offspring per family, and N = the number of families.

This value is then multiplied by 4 to give the variance for the heritability estimate.

(Falconer, 1981, Ch. 10)

Heritability estimates derived from the different methods were then compared.

6.3: Results

6.3:1 Preliminary data description

Table 6.1 presents the mean, standard deviation, coefficient of variation and sample size for the two traits examined. Birds are categorised into groups appropriate for heritability estimates, allowing the sex and age classes of parents and offspring to be examined separately. It can be seen that sample sizes for offspring measured as adults are small for all traits; these data are therefore only used when they can be combined with other offspring data, where no age differences are shown for the traits (see below). Coefficients of variation may be compared between traits; higher values indicate that the trait may be suitable for heritability estimates, as variation among families would in this case be more easily detected. A high values is seen for weight when data are combined from both age classes and sexes ('all birds' of table 6.1), but tarsus length has a relatively low value, indicating that family differences would be expected to be small in the case of tarsus length.

Distributions of metric traits

When sub-divided into the four sex/age classes described in 6.2, all data for both traits follow normal distributions. In addition, when sex and age classes are combined, the combined results follow a normal distribution for both traits. (figs. 6.1-6.2); no differences according to sex and age classes are suggested by these distributions. From a preliminary scan of the distributions, therefore, no differences according to sex or age are apparent. This is investigated further in the following sections.

Table 6.1 Summary statistics for metric characters of barn owls in population 1. Birds are categorised into groups appropriate for heritability estimations; only those for which family data are available are included. SD = standard deviation; CV = phenotypic coefficient of variation (SD / mean). Note that not all set of groups sum to the total number of birds sampled (e.g. all males + all females); this is because (a) not all offspring were assigned a sex, and (b) adults may be parents of both adult and young offspring.

	WEIGHT (g)				TARSUS LENGTH (mm)			
	n	mean	SD	CV	n	mean	SD	CV
all birds	114	363.30	44.21	0.122	101	66.57	2.00	0.030
all males	50	334.72	41.49	0.124	44	66.79	1.96	0.029
all females	54	388.89	28.93	0.074	53	66.51	2.01	0.030
all adults	59	350.39	46.94	0.134	59	66.72	2.06	0.030
all young	55	377.15	36.69	0.097	42	66.36	1.92	0.029
male parents of young offspring	17	306.41	29.57	0.097	17	66.15	2.09	0.032
fem. parents of young offspring	22	391.23	21.81	0.056	23	66.79	1.72	0.023
young male offspring	23	366.74	32.04	0.087	19	67.02	1.62	0.024
young female offspring	22	392.18	35.23	0.090	19	66.01	2.01	0.031
male parents of adult offspring	5	329.40	19.35	0.059	3	68.43	0.32	0.005
fem. parents of adult offspring	4	372.50	33.04	0.089	3	65.78	2.69	0.041
adult male offspring	6	286.75	11.21	0.039	5	66.80	3.08	0.046
adult female offspring	7	379.29	22.52	0.059	8	67.19	2.58	0.038
all male parents	21	309.24	27.27	0.088	20	66.49	2.10	0.031
all female parents	25	388.68	24.48	0.063	26	66.67	1.81	0.027
all male offspring	29	353.17	40.52	0.115	24	67.04	1.85	0.028
all female offspring	29	389.07	32.72	0.084	27	66.36	2.21	0.033

Fig. 6.1 Distribution of weights (g) for barn owls in population 1 for which family data are available; sex and age-classes combined. A normal distribution curve is included for comparison. n = 114

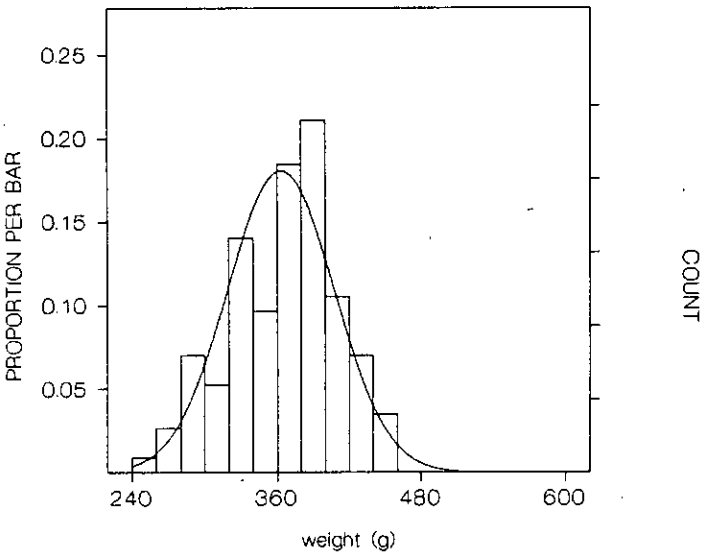
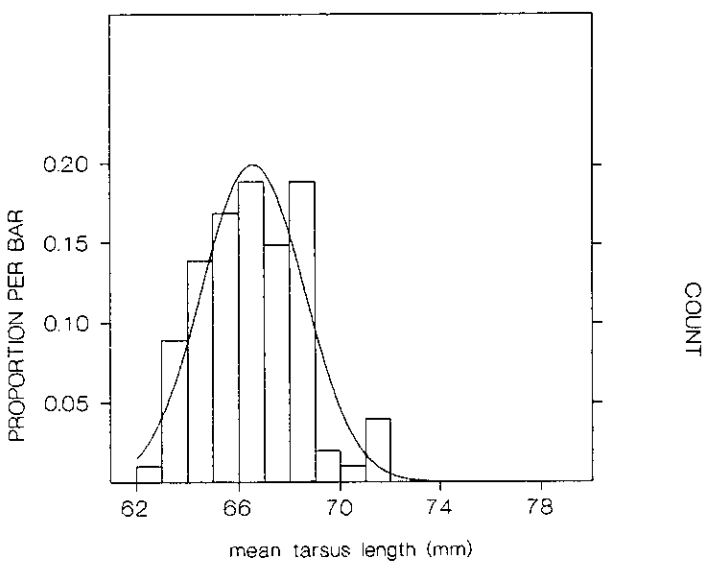


Fig. 6.2 Distribution of mean tarsus lengths (mm) for barn owls in population 1 for which family data are available; sex and age-classes combined. A normal distribution curve is included for comparison. n = 101



Differences in variances between classes

The results of F-tests to compare variances between the categories are shown in table 6.2.

The first two F-tests for each trait in table 6.2 compare variances within age classes (i.e. test for sex differences); the second two tests compare variances within sexes (i.e. test for age class differences), and the last two tests compare variances between both sex and age classes. These tests show weak significance for weight between adult and young females; no other differences in variance are significant. Other differences among the sub-divisions of the data are tested by ANOVA to compare means of the samples.

Comparisons of means

Mean values for the traits were compared by two-way analyses of variance, testing for differences among sex and age classes. The results are shown in table 6.3.

For weight, significant differences between the means are shown for sex, age class, and the interaction between sex and age class; tarsus length shows no significant differences among both sex and age classes.

Implications of the preliminary data description:

(a) Combining data from both sexes

Differences in either mean or variance between the sexes for weight indicate that:

(i) Mid-parent values and mean offspring values should not be used in heritability estimates for weight. They may, however, be used for heritability estimates for tarsus length.

(ii) The sexes should be analysed separately for weight when heritability is estimated by ANOVA of full sibling data.

Table 6.2 Comparing variances between sex and age classes by F-tests, for barn owls in population 1 for which pedigree data were available. F value is calculated as the larger variance / smaller variance in each case, with df_1 = degrees of freedom for the sample with larger variance = (n-1); df_2 = degrees of freedom for the sample with smaller variance = (n-1); significance level = not significant, 0.05 or 0.01 levels of significance. In these tests, 'adult male' is equivalent to 'all male parents' of table 6.1, 'adult female' is equivalent to 'all female parents' of table 6.1, 'young male' is equivalent to 'young male offspring' of table 6.1 and 'young female' is equivalent to 'young female offspring' of table 6.1

Character	Data from:	F	df_1	df_2	significance level
Weight	adult male - adult female	1.24	20	24	ns
	young male - young female	1.21	21	22	ns
	adult male - young male	1.38	22	20	ns
	adult female - young female	2.07	21	24	0.05
	adult male - young female	1.67	21	20	ns
	adult female - young male	1.71	22	24	ns
tarsus length	adult male - adult female	1.34	19	25	ns
	young male - young female	1.54	18	18	ns
	adult male - young male	1.68	19	18	ns
	adult female - young female	1.23	18	25	ns
	adult male - young female	1.09	19	18	ns
	adult female - young male	1.25	25	18	ns

Table 6.3 Results of two-way analyses of variance to test for sex and age differences in the metric traits weight and tarsus length for barn owls in population 1 for which pedigree data are available. STAGE = birds categorised as adult or juvenile.

DEP VAR: WEIGHT N:104 MULTIPLE R: 0.766 SQUARED MULTIPLE R: 0.587

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
SEX	69622.401	1	69622.401	82.251	0.000 ***
STAGE	26750.552	1	26750.552	31.603	0.000 ***
SEX*STAGE	18367.646	1	18367.646	21.699	0.000 ***
ERROR	84645.874	100	846.459		

DEP VAR: TARSUS N: 97 MULTIPLE R: 0.171 SQUARED MULTIPLE R: 0.029

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
SEX	3.955	1	3.955	1.003	0.319 ns
STAGE	0.861	1	0.861	0.218	0.641 ns
SEX*STAGE	7.963	1	7.963	2.020	0.159 ns
ERROR	366.537	93	3.941		

(b) Combining data from adults and juveniles

Differences between age classes for weight indicate that offspring which were sampled when adult should not be included in regression analyses of this trait. With tarsus length adult offspring data may be included in heritability estimates. Age class is not a confounding factor in estimating heritability from full siblings, as only young birds are included in this analysis.

(c) Use of correction factors in estimates of heritability by regression

The correction factor for differences in variance among age and sex classes, as described in 6.2, is used for all the heritability values estimated by regression, for comparison with the uncorrected values. This is shown in 6.3:2.

6.3:2 Estimating heritability by regression

Regressions for the two traits are shown in table 6.4; only in two cases were the regressions between offspring and parent data significant; these were weight (young female offspring - female parent) and tarsus length (mean all offspring - mid-parent). The variances between offspring and parental data were significantly different in 2 of the 9 regressions computed (Table 6.5). For all these regressions, the correction factors for heritability estimates (6.2) were calculated, and are shown in table 6.5.

The heritability estimates derived from these regressions are presented in table 6.6. The most noticeable feature of these estimates is their lack of precision; even the estimates based on a significant regressions between offspring and parental data have large standard errors. The estimate with the lowest standard error is the estimate of 0.64 ∓ 0.27 for heritability of tarsus length from all offspring and mid-parent values.

The 'corrected' estimates illustrate how the unfeasibly large estimates and errors for the uncorrected weight heritabilities may largely be attributed to unequal variances. Use of the correction factor may therefore result in a more meaningful heritability estimate, although the correction should be remembered if direct comparisons between heritabilities calculated by different methods are undertaken. In two cases, the correction factor resulted in a slight increase in the heritability estimate and its error (see table 6.5); this was due to adult variance exceeding the juvenile variance in tarsus length for those sub-sets of data, but the differences in variance were not significant. The effect of the correction factor is therefore small in comparison to cases where there was a significant difference between adult and juvenile variances. An alternative approach to estimating heritability is taken in 6.3:3, and the results of the two approaches compared in 6.4.

Table 6.4 Results of regressions of mean offspring values on parental values for the traits weight and tarsus length for barn owls in population 1 for which pedigree data were available. n = the number of families; m = the gradient of the regression; t = result of two tailed t-test to test significance of the regression between offspring and their parents; sig. = the significance of the regression between offspring and parents- ns = not significant; *p = 0.05-0.01, df = (n-1)

Trait	Regression	n	m	S.E.	t	sig.?
Weight	young male offspring-male parent	9	0.279	0.296	0.942	ns
	young female offspring-female parent	12	0.744	0.324	2.295	*
	young male offspring-female parent	11	-0.354	0.400	-0.885	ns
	young female offspring-male parent	10	-0.590	0.525	-1.123	ns
tarsus length	mean all offspring-mid parent	16	0.638	0.270	2.367	*
	all male offspring-male parent	12	0.354	0.268	1.322	ns
	all female offspring-female parent	15	-0.028	0.371	-0.076	ns
	all male offspring-female parent	14	-0.146	0.389	-0.374	ns
	all female offspring-male parent	13	0.738	0.366	2.015	ns

Table 6.5 F-tests to test for differences between the variances of the parental and offspring data used in the regressions in table 6.5. A correction factor of parent SD / offspring SD is calculated for the heritability estimates (the heritability estimate and SE are multiplied by the correction factors shown here, in table 6.6). sig. = the significance of the difference between the two variances; ns = not significant, *p = 0.05-0.01; **p <0.01

Trait	Data from:	F	df_1	df_2	sig.	correction factor
Weight	young male offspring-male parent	1.17	22	16	ns	0.921
	young female offspring-female parent	2.61	21	21	*	0.619
	young male offspring-female parent	2.16	22	21	*	0.681
	young female offspring-male parent	1.42	21	16	ns	0.840
Tarsus length	mean all offspring-mid parent	1.18	54	45	ns	1.073
	all male offspring-male parent	1.29	19	23	ns	1.135
	all female offspring-female parent	1.49	26	25	ns	0.819
	all male offspring-female parent	1.04	23	26	ns	0.978
	all female offspring-male parent	1.12	26	19	ns	0.950

Table 6.6 Heritability estimates from regressions of offspring on parental data. Regression of mean offspring on mid-parent value gives the heritability directly; single sex estimates and their standard errors are 2X the regression results shown in table 6.4. The estimates and their S.E. are multiplied by the correction factor shown in table 6.5 where parental and offspring variances differed; corrected and uncorrected heritability estimates are shown for comparison.* marks the two regressions in which the regression of offspring on parents was significant in table 6.4.

Trait	Regression	uncorrected h^2 ∓ S.E.		corrected h^2 ∓ S.E.	
Weight	young male offspring-male parent	0.56	∓ 0.59	0.52	∓ 0.54
	young female offspring-female parent*	1.49	∓ 0.65	0.92	∓ 0.40
	young male offspring-female parent	-0.71	∓ 0.80	-0.48	∓ 0.54
	young female offspring-male parent	-1.18	∓ 1.05	-0.99	∓ 0.88
Tarsus length	mean all offspring-mid parent *	0.64	∓ 0.27	0.69	∓ 0.29
	all male offspring-male parent	0.71	∓ 0.54	0.81	∓0.61
	all female offspring-female parent	-0.06	∓ 0.74	-0.05	∓ 0.76
	all male offspring-female parent	-0.29	∓ 0.78	-0.28	∓ 0.76
	all female offspring-male parent	1.48	∓ 0.73	1.41	∓ 0.69

6.3:3 Estimating heritability from full siblings

The heritability estimates based on full siblings are presented in table 6.7. Significant differences in the metric traits among families were shown for female weight. As with the heritability estimates by regression, a lack of precision is noticeable in these estimates.

In summary, the heritability estimates derived from parent-offspring regressions and from full siblings vary according to the method used to calculate them, and all have large standard deviations / errors resulting in few significant results, although correcting for age and sex differences in some estimates gave lower estimates and errors. Lower errors were associated with the regressions of mid-parents than single sex estimates. Possible explanations and implications of these results are discussed in the following section.

Table 6.7 Heritability estimates derived from full siblings by ANOVA, as described in 6.2. Families were restricted to those with data from two or more offspring for each analysis; no offspring sampled as adults were included. The F-ratio and significance levels refer to the significance of the difference among families of offspring in population 1; S.D. = the standard deviation of the heritability estimate, calculated from the heritability variance described in 6.2.

Trait	n families	F-ratio		h^2	S.D.
male weight	8	2.695	ns	0.88	0.57
female weight	6	4.484	*	1.02	0.49
tarsus length	14	1.541	ns	0.30	0.34

6.4: Discussion

Heritability is commonly used as a predictor of the response to selection according to the equation:

$$R = h^2 S$$

where S is the selection differential, the difference in the population mean occurring during one generation under the influence of selection, and R is the predicted response to selection in the next generation (Houle, 1992).

The use of heritability estimates in estimating the response of a wild population to selection in the wild, or to predicting the likelihood of genetic changes occurring in a captive population, is clearly dependent on the precision and bias in the heritability estimates, and this may vary according to the chosen method of calculation. How parent-offspring regressions and sibling analysis compare with respect to precision and bias in this study are discussed below.

6.4:1 Sources of error and bias

(a) Measurement error

Both parent-offspring regressions and full sibling analysis showed low precision in heritability estimates in this study. Measurement error was low when calculated for tarsus length (5.2), indicating that this is not the source of non-significant regressions of parent and offspring data, or of high variances within families, however.

(b) Sample size

Falconer (1981) notes that standard errors are commonly large in heritability estimates unless the regressions or correlations are based on a large number of individuals; in a controlled breeding experiment steps may be taken to achieve suitable numbers as the total number of individuals, and the number of individuals per family can be controlled. In a wild population, such control is not possible, but choices may still be made concerning sample size. In this study, for instance, sampling young barn owls was relatively easy in comparison to catching the adult birds; in similar situations where time or money are the limiting factors for sampling a wild population, heritability estimates based on full siblings may be favoured. Where the total population is small, sample size could be increased by sampling over several years, particularly where individuals may be identified from one year to the next.

(c) Temporal effects

The problem with the above approach is that sampling over several years may increase the environmental components of variation. This effect would be most pronounced for the

characters which have a short development time before reaching a non varying adult size (e.g. tarsus length, Taylor, 1994 12.2), and the most blurred in characters which are more sensitive to immediate environmental fluctuations. Even characters such as weight, however, which show the most short term variation, still include a proportion of their variance as due to permanent developmental effects, and would therefore be influenced by this phenomenon to some extent.

Heritabilities calculated by regression over several years are likely to differ from single year estimates, and especially in a species such as the barn owl which has large annual fluctuations in food availability. The magnitude and direction of the discrepancy will depend on the particular circumstances of each year and family, with the highest heritabilities occurring when the environmental conditions which occurred during parental development match the conditions experienced by their offspring. It follows that a heritability estimate for the whole population is influenced by the proportion of families in which the environmental conditions that year match those of the parents' year of development. Regular, cyclical changes in the environment could therefore introduce non-random patterns of parent-offspring resemblance, which would influence heritability estimates calculated by regression. In a long term study, the effects of several complete cycles on heritability could be assessed, but this was not possible within the time available for this study.

Estimates based on full sibling resemblance would not be affected in the same way; if the data are drawn from a single cohort then no annual environmental variance is involved. In this case, combining data from several years would increase heritability estimates, as the effect would be to enhance the between family component of variation due to different environmental conditions in different years.

Sibling analysis may therefore be preferred to parent-offspring regressions if large annual fluctuations in environmental effects are suspected.

(d) Age effects

A complication involving sampling offspring at the nest arises if sampling occurs before the offspring reach adult size. Sampling offspring in the nest gives samples for which the same environment may be inferred for each family of offspring, and may in any case be the best method of obtaining large samples of known relatives. The problem which arises here, as Boag (1983) found with the beak length of his large cactus ground finch *Geospiza conirostris*, is that offspring may not reach adult size before they fledge; heritability of beak length was low when measured on nestlings in the ground finch.

Published heritabilities are sometimes estimated by a regression of juvenile characters on adult parental values. Aggrey and Cheng (1992), for instance, estimated heritability for pigeon squab weight at weekly intervals up to their marketable age of four weeks, regressing the offspring values onto parental weights; the aim was to assess the possible responses to selection at different ages, to identify whether productivity could be increased by selection. Heritabilities calculated in this way assume a direct correlation between the juvenile character at a given age, and the equivalent adult character. A problem with this analysis arises through the effects of different growth rates among offspring in a family; Atchley (1984) describes how growth rates of young mice change as a function of body size rather than age. Within a cohort, the phenotypic variance is highest at the point where growth changes from exponential to linear; variance decreases as the cohort approaches its adult size. Whatever the final size of the offspring, therefore, each individual is likely to attain it by growing at different rates during development. Compensatory mechanisms allow retarded growth at early stages, such as would be produced by a shortage of food, to be counteracted by later growth. If this model of growth is widely applicable, and Atchley describes similar patterns in guinea-pigs and poultry, the implication for heritability analyses is that traits measured on growing individuals may show greater variance than the parental population even in the absence of selection.

In any case, such heritability estimates are not equivalent to estimates taken from directly comparable datasets of offspring and parents, as the environmental components of the phenotypic variation will vary according to the stage of development. Caution is therefore required when interpreting heritability estimates attained in this way. This is illustrated by Boag's ground finch data (1983); when 53 of the nestlings described above were recaught as adults, heritability of beak length was higher; beak extension continued even after fledging in these birds.

The appropriate age at which to sample a population to obtain accurate heritability estimates therefore depends on the nature of each trait studied. Defining a developing character by age may be inadequate if growth rates among offspring vary; heritabilities calculated from sub-adult offspring and adults relate to their particular circumstances only, and can not be generalised across age groups.

In this study, measurements were taken on offspring in the nest as close to fledging as possible, in an attempt to sample tarsus and wing lengths at adult size. ANOVA between age classes indicates that this was successful for tarsus, but not for wing lengths, and wing length was rejected from this heritability study for this reason (see 5.2).

(e) Assumption: no selection

The effects of selection can be controlled in breeding experiments by breeding from all the progeny in a generation, or from a random sample of individuals. In natural populations, particularly when only a small proportion of the juveniles are recruited to the breeding population, it seems less reasonable to assume an absence of selection. Indeed, it would seem likely that selection on traits related to body size would be common in natural populations, although selection on quantitative traits has rarely been conclusively demonstrated in wild birds, and some such studies have failed to detect selection (Price and Boag, 1987).

It was described above how the heritability estimates from regressions and from sibling analysis would give different results due to the different environmental influences on parents and offspring. Another difference involves the influence of selection. As full sibling heritability estimates are not sensitive to selection in this way, this may be the preferred method of calculation if selection is suspected.

(f) Assumption: Random distribution of genotypes

How realistic is it to assume that genotype and environment are not correlated in any way in natural populations? Non-randomness is introduced by assortative mating, maternal effects or through non-random distribution with respect to habitat quality. Boag and van Noordwijk (1987) cite examples of wild bird populations where these assumptions were met, yet this is not consistently the case; it is not appropriate to generalise between species or populations when they differ in their environmental interactions.

The techniques used to investigate genotype-environment interactions are not feasible for all wild populations; differences in hatching dates, for instance, cause problems with cross-fostering experiments, and there may be ethical reasons for not interfering with a breeding population. Comparing heritabilities calculated by different techniques remains a useful way of investigating the possibility of such interactions, however.

In the case of the barn owl population, high heritabilities from full sibling analysis indicate a common nest environment effect; this may be due to genuine differences in environmental conditions between nests, or be an artefact of the sampling method. If nests were sampled when the offspring were not fully grown, and at different average ages between nests, sibling resemblance would be higher than among family resemblance, resulting in high heritability estimates. Differences between heritability estimates derived from different regressions also

suggest a non-random element in the data, although the large standard errors mask any clear pattern here.

In the absence of any evidence from cross-fostering experiments, how likely is a random distribution of genotypes in the barn owl population?

Habitat quality varies among territories in this population of barn owls, as demonstrated by site productivity over several years (Taylor, 1994). Nest sites are limited, and not all adults breed in any one season. The breeding birds remain in the same location throughout the year, although home range size may show some seasonal variation; establishment of a breeding site occurs in late winter when differences in fitness through food stress would be at their most extreme. If size gives a competitive advantage to nest site competition, and a territory which provides the best food supply during the winter is also the best when it comes to rearing young, the potential for a non-random distribution of genotypes would seem particularly strong in such a population. Given that young barn owls tend to nest adjacent or close to their natal site, this effect could escalate if neighbouring territories were on average more similar to the parental territory than to a random territory in the population; birds with the genetic potential to be large would occupy the best sites.

This effect could be entirely masked by environmental effects, however. The role of maternal effect and territory quality may be very important in a situation as described above, bearing in mind that selection acts on phenotypes, not genotypes. Although it would be predicted that a larger (more competitive) bird would tend to occupy the best territory, this may not be what actually happens in a natural situation. Suppose a new area is colonised, and as there is little competition, a small pair become established on a good territory. Their offspring, although not having the genetic potential to grow as large as others in the population, grow to their full potential and enter their first winter in good condition. Their conspecifics reared in suboptimal conditions do not fare so well, although their genotype may be such that they would be more competitive if they had experienced the benefits of a good territory during their development. They may never regain that lost first advantage; whether their phenotypic size is actually smaller than the others, or due to a general poorness of condition, they occupy the suboptimal territories when they come to breed, and so their offspring are similarly disadvantaged. This effect could continue for generations, with lineages maintaining possession of the optimal territories through environmental effect rather than genetic advantages. This effect would be enhanced by strong site loyalty, and could partly be maintained by the advantage of familiarity which the young birds would have both for the local environment and their neighbours, particularly in cases where an offspring replaces a parent as the breeding bird at its natal site.

All that is required to set this process off is that birds which would normally be at a competitive disadvantage become established on a good breeding site. This is not difficult to imagine if it is remembered that inexperienced breeders have an imperfect knowledge of what constitutes a good breeding and overwintering territory, and that competition in a newly colonised area is likely to be low.

Environment-genotype interactions may therefore be very complex in natural populations, and heritability estimates calculated for populations where these issues have not been explored may be misleading.

6.4:2: Evaluating the heritability estimates

A heritability value is a single figure used to describe a complex system; its value as such in the prediction of a response to selection in breeding experiments is undisputed. It is not always appropriate to simplify a system to such an extent, however; Lewontin and Feldman (1975) describe the dangers inherent in such a simplification. A figure may be used in support of a theory or argument without reference to the way it was derived, yet if calculated by another method or using a different dataset, a quite different figure may emerge. If confidence limits are not also included with the estimate, the scope for misinterpretation escalates. This is seen in the use of heritability values in the ongoing nature/nurture debate about traits such as IQ or diseases in humans, where figures are quoted out of context to support one extreme view or another. It is conceivable that similar problems could arise if heritability estimates were used as a management tool in conservation, if the limitations of what the figure actually represents are not fully described.

The methods by which heritability is commonly estimated were developed for systems where the necessary assumptions were easily met, namely randomness among genotypes and an absence of selection. Family number, size and mating system can all be controlled in breeding experiments, hence the proven success of using heritability values to predict the response to artificial, directional selection, where the magnitude and direction of selection are known and can be manipulated.

The value of using such methods on wild populations depends on how well the assumptions and requirements are met. Boag and van Noordwijk (1987) indicate that heritability estimates on wild bird populations may be compatible with controlled studies; examples are cited where assumptions are not violated and sample sizes are large. This may reflect a bias in published

studies of heritability in wild populations, however, with an understandable tendency to study species likely to reveal good results.

Heritability in this study was estimated because an understanding of the sources of variation in quantitative traits could have management implications, rather than because it seemed likely that the wild barn owl population would yield good estimates of heritability.

Of the two methods used in this study, full sibling analyses avoid biases due to sampling over different years, and sampling offspring in the nest avoids the possibility of selection occurring between fledging and breeding. As birds are usually easier to sample before fledging, this may be the preferred method for estimating heritability, but drawbacks include a common nest environment inflating heritability estimates, and the practical consideration of sampling when the young have attained adult size. Regressions are also sensitive to sampling offspring at sub-adult size, but the common nest environment is not a problem. The use of maximum likelihood techniques would be particularly useful for studies of this kind, as data from all known relations can be incorporated in a single analysis, and if data are included on the year of sampling, natal sites etc. the relevance of these factors can be quantified, given a sufficiently large dataset. The drawback of this approach at present is the difficulty in implementing the analysis, but its popularity may increase in studies of natural populations.

Offspring-parent regressions in this study gave two significant results; the corresponding heritability estimates were (∓S.E.):

- weight: 1.49 ∓ 0.59 (female offspring-female parent) uncorrected estimate
  0.92 ∓ 0.40 (female offspring-female parent) corrected estimate
- tarsus length: 0.64 ∓ 0.27 (mean offspring- mid parent) uncorrected estimate
  0.69 ∓ 0.29 (mean offspring- mid parent) corrected estimate

ANOVA of full siblings gave a significant difference among families for weight (∓S.D.):

- female weight: 1.02 ∓0.49

The heritability estimate for tarsus length based on the regression of mean on offspring-mid parent values is likely to be the best estimate from this study, in terms of precision and bias; the uncorrected value gives the lowest error, as in this case the parental variance exceeded the offspring variance. This difference in variances was not significant, however, and the difference between the corrected and uncorrected estimates is therefore small. The uncorrected estimate is to be favoured for comparison with other studies.

Some studies compare heritability estimates directly among populations or species in an attempt to compare levels of genetic variability in quantitative traits; Cheverud et al. (1994), for instance, compare their heritability estimate for body weight in cotton-top tamarins with a review of heritability estimates for weight and other morphological characters in natural populations, concluding that as their estimate falls within the range of heritability values, there is likely to be a reasonable amount of genetic variation in the tamarin population. A direct comparison of heritability estimates in this way is misleading and inappropriate, however, as heritability is not a measure of the absolute amount of genetic variation in a population, or of the potential 'evolvability' of that population (Houle, 1992). Heritability is a measure of the relative contribution of additive genetic to total variation; it is therefore specific to the population and time for which it was estimated. This is particularly relevant in wild populations, where the environmental component of variance may differ greatly among populations or years. Inter-population comparison is only valid if the actual amount of phenotypic variation is taken into account in the comparison. This requirement is met by the coefficient of genetic variation:

$$CV_A = 100 * (S.D. / mean) * \sqrt{h^2}$$

(Houle, 1992)

and it is therefore proposed in this study that any comparative study of the genetic basis of variation in quantitative traits should use CV_A as the means of comparison. Unfortunately, many published heritability studies neglect to include the basic summary statistics (phenotypic mean; standard deviation or variance) which would allow CV_A to be calculated. Table 6.8 presents CV_A data from several avian studies for comparison. Genetic variability of barn owl tarsus length as assessed by the genetic coefficient of variation ($CV_A = 2.40$) lies in the middle of the range of these published values; it does not appear significantly lower in *T. alba* than for populations in which genetic adaptation for quantitative characters has been demonstrated in the wild (Boag, 1983). There is therefore no indication in this self-sustaining population of *T. alba* that levels of quantitative variation are too low to permit future genetic adaptation for tarsus length; the population may still maintain sufficient genetic variation in quantitative traits to adapt in a changing environment. The implications for conservation management of finding high and significant tarsus length heritability in this study are discussed in chapter 8.

Table 6.8 Summary statistics and heritability for tarsus length from this study of *Tyto alba*, and for other published avian studies. CV_P denotes phenotypic coefficient of variation 100 *(S.D. / mean); CV_A denotes genetic coefficient of variation $CV_P*\sqrt{h^2}$. The studies are ranked in order of decreasing CV_A

species	sample	n	mean ±S.D.	CV_P	h^2 regression	n broods	h ±SE	CV_A	Source
Geospiza scandens			20.60	3.64	mid-parent, mean offspring	16	0.92 ±0.23	3.49	Boag (1983)
Geospiza conirostris	offspring	28	22.05 ±0.78	3.54	mid-parent, mean offspring	20	0.78 ±0.23	3.13	Grant (1983)
Hirundo rustica	adult females	659	12.15 ±0.68	5.60	female parent, mean offspring	659	0.27 ±0.05	2.91	Moller (1989)
Geospiza fortis	adults	44	18.75 ±0.64	3.42	mid-parent, mean offspring	39	0.71 ±1.00	2.88	Boag (1983)
Hirundo rustica	adult males	712	12.16 ±0.71	5.84	male parent, mean offspring	464	0.20 ±0.05	2.61	Moller (1989)
Ficedula hypoleuca	adult females	715	19.49 ±0.47	2.41	female parent, mean offspring	167	1.02 ±0.14	2.44	Alatalo et al. (1990)
Tyto alba	all adults and juveniles	101	66.57 ±2.00	3.00	mid-parent, mean offspring	16	0.64 ±0.27	2.40	This study
Ficedula hypoleuca	adult females, Trondheim popn.	69	19.49 ±0.53	2.72	female parent, mean offspring	69	0.58 ±0.15	2.07	Lifjeld & Slagsvold (1989)
Ficedula hypoleuca	adult females, Oslo population	150	19.54 ±0.47	2.41	female parent, mean offspring	150	0.62 ±0.13	1.89	Lifjeld & Slagsvold (1989)
Ficedula albicollis	adult females	916	19.52 ±0.49	2.51	mid- parent, mean offspring	919	0.52 ±0.04	1.81	Alatalo et al. (1990)
Sturnus vulgaris	adult females	298	33.99 ±0.74	2.18	female parent, mean offspring	136	0.24	1.09	Smith (1993)

SUMMARY

Heritability estimates for the metric traits weight and tarsus length were derived for barn owls from a wild population in south west Scotland. Two methods were compared: regressions of offspring on parents, and analysis of variance for full siblings. Both methods involved data from birds of known pedigree which had been assigned a sex according to their breeding records or plumage markings, as described in chapter 4.

All estimates were characterised by large standard errors; significant results were:

(a) Estimates from offspring-parent regressions (∓S.E.):

- weight: 1.49 ∓ 0.59 (female offspring-female parent) uncorrected estimate
  0.92 ∓ 0.40 (female offspring-female parent) corrected estimate
- tarsus length: 0.64 ∓ 0.27 (mean offspring- mid parent) uncorrected estimate
  0.69 ∓ 0.29 (mean offspring- mid parent) corrected estimate

(b) ANOVA of full siblings (∓S.D.):

- female weight: 1.02 ∓0.49

Of these estimates, the weight regression was based on data with unequal variances between parents and offspring. The full sibling figures may be overestimates due to families being sampled at different ages, and hence the among family component of variance being inflated.

The heritability estimate for tarsus length based on the regression of mean offspring on mid parent values is likely to be the best heritability estimate from this study, in terms of precision and bias.

It is proposed in this study that any comparative study of the genetic basis of variation in quantitative traits should use CV_A as the means of comparison. CV_A for tarsus length in this study was 2.40; this lies in the middle of the range of the published avian values reviewed here.

Chapter 7

PROTEIN ELECTROPHORESIS

7.1: Introduction

In contrast to the quantitative traits discussed in chapters 5 and 6, the technique of protein electrophoresis offers the opportunity to generate population genetic data which are relatively easy to interpret, as the variation is under the control of few genes with little environmental influence. Unlike quantitative data, the results from electrophoresis trials can in most cases be interpreted directly as genotypes, allowing interpopulation and interspecific comparisons uncomplicated by unknown numbers of loci influencing a particular trait, or by questions of heritability. Such direct sampling of transcribed portions of the genome is potentially a very powerful tool in population genetic studies and for conservation management; appropriate application of protein electrophoresis data depends on the rate at which proteins come to differ in electrical charge, whether the changes are subject to selection or are selectively neutral, and whether the variation is representative of genetic variation occuring throughout the genome. These issues have been the subject of much debate since protein electrophoresis became a popular tool in population genetics in the late 1960s, and are discussed in this chapter, to identify the relevance of this widely applied technique to conservation. Electrophoretic variation in proteins is then described for the five barn owl populations in this study.

7.1:1 Principles of protein electrophoresis

Protein molecules consist of strings of amino acids, some of which have charged side chains; lysine, arginine and histidine have positively charged side chains, whereas aspartic acid and glutamic acid have negatively charged side chains (Avise, 1994). A protein molecule therefore carries a net charge, which will vary according to the pH of the medium to which it is exposed. Samples of soluble proteins derived from blood or tissue may be separated according to their net charge by passing an electric current through a supporting medium such as a starch or polyacrylamide gel, and their position detected by the use of a specific histochemical stain. Each stain consists of a buffer solution of the appropriate pH, the enzyme's substrate, any necessary co-factors for the reaction, and an oxidised salt. At the position in the gel where the particular enzyme has migrated, the ensuing reaction reduces the salt to produce a coloured precipitate, which appears as a band on the gel. The resulting banding pattern, the zymogram, represents all the electrophoretic variants of the specified protein. A single band is diagnostic of a homozygous individual; multiple bands may represent heterozygotes or homozygotes at more than one locus (Evans, 1987). The intensity and position of the bands, combined with a knowledge of the proteins quarternary structure, allows individuals to be

scored as heterozygous or homozygous, and alleles are recorded by specific codes according to their distance migrated through the gel. Post translational changes to proteins may confuse the interpretation of banding patterns, but the genetic basis of the bands can be tested for expected patterns of Mendelian inheritance. Data from both parents and offspring are therefore desirable in a population study, although in some cases confirmation is possible by manipulating the breeding of tested individuals, and subsequently testing of their progeny. The different molecular forms of an enzyme or protein, whether genetic or non-genetic in origins, are termed 'isozymes', whereas a unique electrophoretic form of an enzyme or protein determined by a particular allele is termed an 'allozyme' (Strickberger, 1976).

Analysis of protein electrophoretic data

Once the genetic basis of the banding patterns of a zymogram has been confirmed, individual heterozygosity across all the proteins tested can be calculated, and the mean population heterozygosity (H) can be derived by pooling the data from all individuals in a population. Similarly, the level of polymorphism (P) for a population can be determined, where a locus is termed polymorphic if the frequency of the most common allele is less than 0.95 (although some studies use a 0.99 cut off point) (Evans, 1987). The frequency and distribution of alleles has been used to derive measures of genetic identity and genetic distance, the most commonly used being Nei's index of genetic identity (Nei, 1978), which describes the probability that alleles drawn at random from two populations will be identical. Wright' F-statistics (Wright, 1965) test for genetic heterogeneity, reflecting the extent to which a population is divided into sub-populations with restricted gene flow.

7.1:2 Variation revealed by protein electrophoresis

(a) Heterozygosity and polymorphism

Early electrophoresis studies revealed high levels of genetic variation; studies of 20-50 loci in humans and *Drosophila* gave levels of polymorphism of 30% or more, with around 10% of loci heterozygous in each individual (Avise, 1994). A review of protein electrophoresis variation in natural populations, representing studies carried out up to 1976 which covered 14 or more loci, found taxonomic differences in levels of P and H; P was estimated at 0.147 (SD 0.098) for 46 species of mammals, 0.219 (SD0.129) for 17 species of reptiles, and 0.150 (SD 0.111) for 7 species of birds. The corresponding estimates of H were 0.0359 (SD 0.0245) for mammals, 0.0471 (SD 0.0228) for reptiles and 0.0473 (SD 0.0360) for birds (Nevo, 1978). This review demonstrated that Aves were under-represented in early isozyme studies, with only 10 of 8600 species from 2 of the 28 orders having been studied, compared, for instance, to 15 of 6300 reptile species from 1 of 4 orders, and 46 of 3700 mammal species from 3 of 19 orders (Nevo, 1978); generalisations about levels of variation among taxa are therefore

questionable from these data. Wild avian studies became more widespread in the late 1970s and 1980s, however, and a review of 30 avian isozyme studies by Barrowclough, involving populations of 17-269 individuals and 14-44 loci (Barrowclough, 1983, cited in Evans, 1987) gave an estimate of H at 0.053; Corbin (1983) covering 71 bird species gave H as 0.0673, and a P estimate of 0.222. Evans (1987) reviews these and additional studies published up to 1984, covering 5-1414 individuals per species and 14-46 loci; mean H over 86 species was 0.044; P from 103 species was 0.240; it was concluded that in general, birds have slightly higher estimates of P and H than mammals.

Within the Strigiformes, Radler (1992) found two of eight enzymes polymorphic in a study of eagle owls *Bubo bubo*, giving a polymorphism estimate of 0.25; Barrowclough and Gutierrez (1990) found no isozyme variation in 6 of 7 populations of the Spotted owl *Strix occidentalis*. Randi et al. (1991) present data on allele frequencies at 28 loci for seven species of owl from which polymorphism can be estimated for each species; the estimates are shown in table 7.1, where P (0.95) represents loci which are considered polymorphic if the frequency of the most common allele is 0.95 or less, and P (0.99) represents loci which are considered polymorphic if the frequency of the most common allele is 0.99 or less.

Table 7.1 Polymorphism in the Strigiformes calculated from allele frequencies presented by Randi et al. (1991); n = number of individuals

Species	n	P (0.95)	P (0.99)
Tyto alba	19	0.0714	0.0714
Athene noctua	17	0.1429	0.1429
Strix aluco	16	0.0714	0.0714
Otus scops	2	0.0000	0.0000
Asio otus	18	0.0714	0.2143
Asio flammeus	3	0.0714	0.0714
Bubo bubo	2	0.0714	0.0714

These estimates appear lower than the average level of polymorphism described for birds, but such generalisations may not be particularly meaningful. Small sample sizes contribute to underestimates of polymorphism, and the selection of enzymes assayed affects the results, as some tend to be more polymorphic than others. Given that species may also exhibit variation in their levels of polymorphism and heterozygosity among populations (Evans, 1987), only studies which estimate variation in P and H in several populations of each species allow interspecific comparisons to be carried out with any confidence; a large number of randomly

selected loci and a large number of individuals per population are also desirable. This type of data is not easy to acquire for wild populations of birds, and so the extent of isozyme variation within and among populations remains incompletely described in the majority of studies.

(b) Genetic distances

Where isozyme variation has been studied at different taxonomic levels in birds, Nei's genetic distances among different taxonomic levels suggest that birds show the least differentiation compared to mammals and to *Drosophila*. Genetic distances of around 0.2, which are associated with differences at the sub-species level in mammals and *Drosophila*, are comparable to differentiation at the level of genera in birds (Evans, 1987). Avise (1994) summarises population differences in vertebrate and invertebrate taxa from protein electrophoresis studies of 321 animal species; birds show the lowest differentiation among populations, which Avise suggests is due to the high mobility of birds compared to other taxa.

Within the Strigiformes, Randi et al. (1991) calculate a Nei's genetic distance of 1.49 between *Tyto alba* and the Strigidae, and among the Strigidae, D= 0.88, distances far greater than the distances usually reported for birds. Randi et al. suggest this discrepancy is due to different studies using different sets of loci, as D is strongly influenced by the choice of loci.

Although differentiation among local populations has been demonstrated by protein electrophoresis in birds (Bacon, 1979; Corbin, 1983; Evans, 1987), low expected levels of differentiation among bird populations suggest that inter-population comparisons should cover a large number of loci, involving as many individuals as possible in each population, if population differences are to be demonstrated.

7.1:3 Interpretation of protein electrophoretic data

The above review gives an idea of the extent of protein electrophoresis variation found in wild bird populations, but simply describing patterns of heterozygosity, polymorphism or genetic distances is inadequate if practical use is to be made of this type of data in conservation. Only if the processes which cause the observed results are understood can data be interpreted in any meaningful way, and so it becomes relevant to discuss whether protein electrophoretic variation is representative of the whole genome, and to discuss by what processes the variation is maintained.

(a) How representative is protein electrophoresis data of the whole genome?

(i) Hidden variation

Only around 10% of the genome codes for proteins; of these, electrophoresis is only possible for the water soluble ones, and appropriate stains have only been developed for around 100 of

these. Of the proteins commonly assayed by electrophoresis, there is a strong bias towards the enzymes involved in certain metabolic pathways, and a preference for using the cheaper stains where possible. Of the loci which are sampled, the genetic variation detected is not comprehensive; only substitutions which result in a net alteration to the proteins charge are detected, yet studies in the 1970s suggested that this variation could be only the 'tip of the iceberg' with many more alleles present than could be detected on the basis of electric charge alone. Marshall and Brown (1975) modelled the effect of single base mutations on the resultant charge of a protein, and concluded that 23% of all single base mutations would represent synonymous mutations, and that only 25% of single base substitutions, or 33% of amino acid substitutions, would result in a charge change in the protein molecule. This was first tested experimentally by Singh et al., (1976) by sequential gel electrophoresis (SAGE). In this technique, electromorphs of polymorphic proteins determined by conventional electrophoresis to be identical were retested under different conditions of pH, buffers and pore sizes, which revealed otherwise undetected alleles. Whereas conventional electrophoresis detected eight alleles for xanthine dehydrogenase (Xdh) in *Drosophila pseudoobscura*, for instance, SAGE revealed 27 alleles, most of which occured at low frequency. Testing SAGE and conventional electrophoresis on the known variation of sequenced myoglobin and hemoglobin gave a detection rate of 40% of known substitutions for conventional electrophoresis, compared to 93% and 86% for SAGE (Lewontin, 1985). When the same technique was applied to five conventionally monomorphic proteins in *Drosophila*, however, no such hidden variation was revealed, suggesting that although the extent of variation in polymorphic loci is underestimated by conventional electrophoresis, hidden variation is not prevalent in proteins classed as monomorphic by single electrophoresis trials. Levels of heterozygosity detected by a single electrophoresis trial are therefore likely to be underestimates of the true levels of heterozygosity for the loci in question, but generalisations about the proportion of polymorphic proteins in a population are not likely to be biased.

(ii) Correlating protein variation with other methods of assessing genetic variation

It appears that protein electrophoresis samples a small and non random proportion of the genome, and that not all of the variation present is detected by conventional techniques. It should come as no surprise that a lack of correlation is sometimes found between estimates of variability based on protein electrophoresis and on other techniques; different parts of the genome evolve at different rates according to whether they are subject to selection or are selectively neutral, and so past and present demographic events have varying degrees of influence on present day observed levels of variation. Hence Schaeffer et al. (1987) found higher levels of restriction-site polymorphism in *Drosophila pseudoobscura* populations than in *D. melanogaster* populations for the Adh region, although isozyme studies had shown

roughly equivalent levels of protein polymorphism in the two species; a lack of correlation between isozyme variation, monoterpenes and chloroplast DNA, was found in populations of Scots pine *Pinus sylvestris* (Helgason, 1993). Extremely low heterozygosity and polymorphism, as assessed by allozymes, was recorded by Hillis et al. (1991) in Florida tree snails *Liguus fasciatus*, contrasting with high levels of morphological variation in their shells, whereas two species of perennial herbs *Datisca cannabina* and *D. glomerata* are morphologically similar, yet are highly divergent at isozyme loci (Crawford et al., 1992). Ferguson et al. (1991) obtained different estimates of population differentiation based on allozyme and mitochondrial DNA variation in populations of brook char *Salvelinus fontinalis*; clearly variation assessed by one method does not necessarily reflect variation as assessed by other methods, and extrapolation without an understanding of the underlying processes could lead to confusion.

(b) The selection/neutrality debate

As protein electrophoresis data may be a poor indicator of variation present in other parts of the genome, it should not be used in isolation as a measure of overall genetic variability as described by heterozygosity or polymorphism. It certainly should not be used on an individual level to selectively breed for variability, as such artificial selection in favour of certain protein variants could result in the overall loss of variability (Lande and Barrowclough, 1987). What, then, are appropriate applications for this relatively cheap and easily obtained population genetic data? The answer depends on the amount of variation present at different levels of population or taxonomic differentiation, of the rates of evolution and the processes which mediate the changes. Rapidly evolving areas of the genome provide sufficient variation among individuals to answer questions of paternity, whereas particularly conserved regions may be better suited to comparisons among divergent taxa. Rates of molecular evolution are influenced by their susceptibility to selection, and the strength and duration of selection pressures; the interpretation of genetic data is therefore dependent on an understanding of the processes which caused the observed patterns of variation.

If isozyme variation was neutral, it could be used to give information on isolation, migration, ancestry and population size, whereas if it was influenced by selection, it would indicate environmental or life history adaptation (Utter, 1991). This issue has been the subject of intense debate since electrophoresis first revealed the extent of protein variation, with observational, experimental and theoretical evidence cited to support both extremes.

Clines of environmental conditions have commonly been correlated with isozyme frequencies, usually among invertebrates. Zera (1987), for instance, notes the correlation between

environmental temperature and the frequencies of PGI alleles in water striders *Limnoporus canaliculatus*, mussels *Mytilus edulis* and sea anemones *Metridium senile;* it was suggested that the locus is influenced by selection either directly, or through close linkage to other loci. Correlations between genotype frequencies and particular environmental conditions rarely provide convincing evidence in favour of or against selection occuring in natural populations, however, as the effects of other factors which influence gene frequencies usually remain unknown. Only if particular requirements were met could purely observational data be taken as evidence for selection on isozymes in natural populations, for instance if two genetically similar populations became isolated, but retained identical environmental and demographic conditions, they may be expected to diverge in isozyme frequencies over many generations by chance events if the loci were selectively neutral, but would remain similar if the loci were responding to the same selection pressures. These requirements may appear extremely unlikely in any natural event of isolation, yet remarkably this may be the situation experienced by discrete but sympatric populations of pink salmon *Oncorhynchus gorbuscha* (Aspinwall, 1974). In this salmon, even and odd year populations spawn in the same river, but never interbreed. Aspinwall found that sub-populations from one year class but different parts of the river system were more similar in isozyme frequencies than the sub-populations of the other year class, and therefore concluded that the isozymes screened were selectively neutral.

Correlations between genotypes and life history traits have also been sought; the evidence for selection occuring is strongest where the physiological function of the enzyme is known, and can be related to the observational data. Radler (1992), for instance, studied the differential survival of genotypes of captive bred eagle owls *Bubo bubo* released in the wild in Germany, and found a lower mortality of heterozygous EST individuals where death was caused by sickness or injury, but not when the cause of death was electrocution at pylons. The owls were equally likely to perch on pylons, but less fit individuals succumbed to sickness or frequented sub-optimal roadside habitat, hence it was argued that selection occured at the esterase locus in the absence of electrocution. Two further avian examples of possible selection on isozyme loci both involve esterases; Evans (1987) found a correlation between isozyme frequency, clutch size and laying date in British populations of starlings *Sturnus vulgaris.* Homozygotes for the common allele exhibited the highest fecundity; heterozygotes for one rare allele laid clutches later in the season, and were more frequent in the north of their range, whereas the heterozygotes for another allele laid smaller clutches earlier in the season, and were prevalent in the south of the range. This was interpreted as a polymorphism maintained by selection in favour of the different genotypes according to whether the season was early or late, with it usually occuring later in the north. Bacon (1979) also noted differences in clutch size and laying date in mute swans *Sygnus olor*; in this case the EST homozygous females produced

produced large, early clutches, and females mated to heterozygous males produced larger clutches irrespective of laying date, which Bacon attributed to differences in habitat quality. Evans (1987) and Radler (1992) both postulate that it is the esterase which is directly involved in mediating the observed fitness differences, assuming that the function of the enzyme is to break down fatty acids. In the case of the starlings, homozygous nestlings grew well on a diet of poultry meal (a food source utilised by the wild birds late in the season when the preferred invertebrates were in short supply), but heterozygotes were found to be incapable of digesting it, and suffered high mortality. Radler noted that the ability to digest fat, and to mobilise fat reserves, would be a particular advantage for a predatory bird which may be subjected to critical periods of food shortage.

Correlations between genotypes and environmental gradients or life history traits must remain circumstantial evidence for selection or neutrality without experimental evidence concerning the biochemical properties of allozymes, their physiological consequences, and an understanding of how the physiological differences may be translated into fitness differences (Zera, 1987). Zera studied the thermal stability of PGI derived from water striders collected from different geographical areas, and found that the allozymes were significantly different in their properties, and that this correlated with the predictions based on the geographical distribution of the genotypes. Similar correlations for PGI and for other enzymes, are found in other taxa (Zera, 1987). Powers et al. (1991) conducted a thorough investigation into the properties of allozymes in the teleost *Fundulus heteroclitus*. This species ranges across one of the steepest aquatic thermal gradients in the world, on the east coast of North America, experiencing a range of temperatures from below freezing to over 40°C, varying with season and latitude. Activity and thermal stability of the allozymes, and developmental rates, hatching times and swimming ability of the genotypes, were consistent with expectations based on the observed distribution of genotypes in the wild, again supporting the theory that selection may act directly on allozymes.

As it has been shown that there is good observational and experimental evidence that selection acts on allozymes at least some of the time, it then becomes relevant to inquire how common this phenomenon is likely to be. One approach is to consider common natural levels of variation, and to estimate mathematically whether observed levels are likely to be maintained by selection or random change. Lande and Barrowclough (1987) calculated that for a mutation rate of 10^{-5} to 10^{-7} per locus per generation, effective population sizes of at least a quarter of a million individuals would be required to maintain intermediate or high levels of heterozygosity for neutral alleles, a situation which must hardly ever be met in natural populations of animals. Does this mean that high heterozygosity is therefore indicative of

selection? This question can not be answered without demographic evidence; high levels of heterozygosity, for instance, occur if two previously isolated populations in which different alleles have reached fixation then come together and interbreed.

Isozyme variation may therefore sometimes be subject to selection, but the extent and relevance of this phenomenon is not simple to assess. The effects of random drift may outweigh all but the strongest selection pressures in smaller populations; a large proportion of mutations which are slightly deleterious will be effectively neutral in a small population, although they would be selected against if the population were sufficiently large that the effects of random drift were counterracted (Kimura, 1983). In relatively small natural populations (with an effective population size in the region of 10s and 100s rather than 1000s) isozyme variation may therefore often be treated as if it were effectively neutral, even if selection is known to be possible. Lande and Barrowclough (1987) estimate that if neutral single locus variation was largely lost through inbreeding during a severe bottleneck, it would take many generations (10^5 to 10^7) to recover former levels of heterozygosity, whereas quantitative variation could recover an order of magnitude more rapidly. The isozyme variation in a single population sampled on one occasion is therefore not particularly informative if treated in isolation; a lack of variation may be due to recent inbreeding, for instance, or due to a severe bottleneck in the distant past. Depending on the mating system, close inbreeding may not be an immediate problem, as a population with a long history of such matings will have a low genetic load, and so the instant deleterious effects of inbreeding depression would not be observed. If the bottleneck was a recent phenomenon, it may be expected that variability would be low at all levels; if it occured thousands of generations ago, however, other levels of variation may have recovered more rapidly, and so a lack of isozyme variation would not correlate with other measures.

7.1:4 The application of protein electrophoretic data

It has been shown that isozyme variation is not representative of all the genetic variation present in an organism, and that although it may be subject to selection, it can often be treated as if it were neutral as in small natural populations the effects of drift often predominate. The strength of isozyme data therefore lies not in the calculation of absolute values of genetic variation in a particular species, but in monitoring differentiation and change among populations or species.

It may, for instance, provide information on past demographic events in a particular species; low levels of isozyme variation could indicate a population bottleneck, which may have occured many generations ago. This is thought to be the case with the cheetah *Acinonyx*

jubatus, which exhibited no variation in 52 blood proteins surveyed (Cohn, 1986). Interpretation of isozyme population data may be greatly facilitated by the inclusion of other data such as genetic variation assayed by another technique, or known demographic parameters. Hence in the case of the cheetah, variation in mDNA suggested a post-bottleneck recovery time of 6 000 -20 000 years (Menotti-Raymond and O'Brien, 1993); without a combination of approaches, the timescale would remain unquantified.

Present day population structure may also be revealed by protein electrophoresis, hence regional differences in allele frequencies indicate restricted gene flow among sub-populations, as with the European starling *Sturnus vulgaris* (Evans, 1987); the introduction of individuals with rare neutral alleles to a population could be used to demonstrate the rate of gene flow.

Isozymes may also be used as diagnostic markers of species or populations; as diagnostic markers of species, isozymes have been used to monitor hybridisation in morphologically similar species such as red and Sika deer *Cervus elaphus* and *C. nippon nippon* (Abernethy, pers. comm.). Isozyme data allowed the species of a haul of rockfish of the *Sebastes* genus, caught by a Japanese trawler in waters under U.S.A. jurisdiction, to be identified as illegally caught *S.alutus*; at the population level, isozyme data showed that a catch of king crab *Paralithodes camtschatica* which it was claimed came from an area where fishing was permitted, in fact originated from a closed fishing area in the Bering Sea (Utter, 1991).

Protein electrophoresis data are most informative at the level of populations of the same species, and among closely related species (Avise, 1994), yet information on parentage can be derived from the inheritance patterns of polymorphic loci, as with indigo buntings *Passerina cyanea* (Westneat et al., 1987); in some cases it may provide information on individual identity. At the other extreme of taxonomic levels, the differences among distantly related species are too great to allow a meaningful comparison of isozyme frequencies (Avise, 1994).

Protein electrophoresis data therefore have a range of potential applications, from identifying individuals, testing parentage, describing population differentiation and assessing species divergence. Information may be attained about past demographic events and present population structure; analyses are strengthened by the inclusion of complementary data about genetic variation assayed by other methods, present patterns of breeding and dispersal, and historical data about population trends.

7.2: Protein electrophoresis of barn owl samples

The aim of this study is to investigate variation within and among barn owl populations. The two adjacent wild populations could provide data on population differentiation, reflecting gene flow between the populations; the inclusion of the captive populations allows the investigation of possible inbreeding in the captive populations, or the inclusion of alleles not found in the native stock, which could occur if non-native birds were used as breeding stock. The results of protein electrophoresis can be interpreted in the light of known population parameters (chapter 2) and variation in quantitative traits (chapters 5 and 6).

Samples from a relatively large number of birds from more than one wild population are not common in the literature, and Strigiformes are not well represented by isozyme studies (Randi et al., 1991), but the available data suggest low levels of population differentiation among bird populations in general. Screening a large number of loci is therefore desirable, if population differences are to be detected. The development of isozyme systems for barn owl blood samples is described in the following section.

7.2:1 Developing isozyme systems

Clear bands on a zymogram are only produced under particular conditions of gel pH, buffer pH, current and duration of trial; the correct conditions for a particular protein vary according to the source of the samples (eg. blood, tissue), the species, and according to details of the procedure peculiar to a researcher or laboratory. The preliminary stages of a protein electrophoresis study therefore involve the development and refinement of systems to give clear and repeatable results.

On the assumption that blood protein samples from closely related species would behave in a similar manner, initial trials were undertaken using systems developed on eagle owls *Bubo bubo*, which were themselves modifications of the systems developed by Harris and Hopkinson (1976) for human samples of blood and tissue. In January 1990, I received training in isozyme techniques under the supervision of Dr. Karl Radler at Goettingen University, Germany, where eagle owl blood samples were routinely being screened for 18 different proteins. A small number of fresh barn owl blood samples were obtained from a local captive breeder, and these were included on the eagle owl gels to directly test the suitability of these running conditions. The proteins tested are listed in table 7.2.

Table 7.2 Enzymes for which successful electrophoresis conditions have been developed by Radler (1992) for eagle owl (*Bubo bubo*) blood samples

Enzyme	Abbreviation	Enzyme commission (E.C.) number
Glyceraldehyde phosphate dehydrogenase	GAPDH	1.2.1.12
Isocitrate dehydrogenase	ICD, IDH	1.1.1.42
6- phosphogluconate dehydrogenase	6-PGD	1.1.1.44
Malate dehydrogenase	MDH	1.1.1.37
Lactate dehydrogenase	LDH	1.1.1.27
Glucose-6-phosphate dehydrogenase	Gd	1.1.1.49
Red cell esterase	EST-E	3.1.1.1
Plasma esterase	PE, EST-S	-
Adenosine deaminase	ADA	3.5.4.4
Glutamate oxaloacetate transaminase	GOT	2.6.1.1
Peptidase	PEP	3.4.11
Nucleoside phosphorylase	NP	2.4.2.1
Hexokinase	HK	2.7.1.1
Glyoxalase-1	GLO-1	4.4.1.5
Phosphoglucomutase	PGM	2.7.5.1
Glucose phosphate isomerase	GPI	5.3.1.9
Superoxide dismutase	SOD	1.15.1.1
Catalase	CAT	1.11.1.6

Of the 18 eagle owl systems, only three (6-PGD, MDH, LDH) gave any banding at all with barn owl blood, and even these were not as clear as the eagle owl results on the same gels. Staining activity of the other enzymes was tested by spotting some of the blood sample onto filter paper and incubating this in the staining solution without electrophoresis; the production of the characteristic stain colour on the paper was taken as an indication of enzyme activity, and this demonstrated the presence of the other enzymes in the barn owl blood samples. It was therefore concluded that the eagle owl systems were not particularly suitable for barn owl blood, and that different systems, or modifications of the existing systems, would be necessary if good results were to be obtained for barn owls.

Barn owl blood for the development of the isozyme systems was obtained initially from the Hessilhead Wildlife Centre, Beith, Ayrshire, and from ITE Monks Wood Experimental

research Station, Huntingdon, to conserve the samples collected from the target populations during the early stages of the project.

Trials for a number of enzyme systems were undertaken, using amongst others, the protocols reviewed in Evans (1987) developed for a variety of avian species, and Harris and Hopkinson (1976) protocols for human samples. Systems were selected initially on the basis of the cheapness of the stain components, and not according to whether they were expected to be polymorphic. Moderate separation times and voltage were used in the first trials of a system (eg. 40mA for 3-4 hours), the aim being to first establish clear bands, and then to develop conditions which spread them out sufficiently to be unambiguous. Where staining appeared as a smear rather than as bands, subtle adjustments to the pH of the gel or bridge buffers were attempted to improve the resolution.

The development of the isozyme systems was impaired by the accidental degradation of the blood samples collected for trials in 1990 due to a faulty freezer used for the temporary storage of the samples during the relocation of the laboratories; the samples had apparently been repeatedly thawed and frozen over a period of days or weeks. As the problem was not immediately detected, trials were continued on these samples; typically enzyme activity (if present) appeared very streaky on the gels, irrespective of changes in pH and buffer composition, and no progress was made in developing isozyme systems until new samples were used.

Satisfactory electrophoresis conditions were found for 15 proteins which were subsequently used for the routine screening of all samples; the proteins are listed in table 7.3 together with their quarternary structure, where known, to aid in zymogram interpretation.

The successful combinations of buffer solutions and running conditions are presented in tables 7.4 and 7.5; they represent modifications of other avian systems (Radler, pers. comm.; Evans, 1987) with the exception of SOD (a bryophyte system- Hofman, 1991), and MPI (a deer system- Abernethy, pers. comm.). Staining recipies are presented in table 7.6, and are derived from the same sources as the buffers.

The details of the procedure for starch gel electrophoresis are described in the following section.

Table 7.3 Proteins from barn owl blood samples routinely screened by starch gel electrophoresis in this study. After Evans (1987) and May (1992).

Protein	Abbreviation	Source	Enzyme Commission (EC) number	No. of sub-units
Transferrin	Tf	serum	-	1
Non-enzymatic protein	Pt	serum	-	1,2
Albumen	ALB	serum	-	2
Acid phosphatase	ACP	serum	3.1.3.2	2
Leucine aminopeptidase	LAP	serum	3.4.11.1	1
Serum esterase	EST-S	serum	-	1,2
Ng	Ng	serum	-	
Phosphoglucomutase	PGM	red cells	5.4.2.2	1
Glucose phosphate isomerase	GPI	red cells	5.3.1.9	2
Malate dehydrogenase	MDH	red cells	1.1.1.37	2
Lactate dehydrogenase	LDH	red cells	1.1.1.27	4
Superoxide dismutase	SOD	red cells	1.15.1.1	2
Haemoglobin	Hb	red cells	-	1
Mannose phosphate isomerase	MPI	red cells	5.3.1.8	1
6-phosphogluconate dehydrogenase	6-PGD	red cells	1.1.1.44	2

Table 7.4 Effective buffer systems for starch gel electrophoresis of barn owl blood samples.

Protein	bridge buffer	gel buffer
Tf, Pt, ALB ACP LAP EST-S, Ng	0.3M boric acid, 0.01M NaOH pH 8.2	0.05M tris, 0.08M citric acid pH 8.4
PGM GPI MDH LDH 6-PGD	0.15M tris, 0.005M citric acid pH 7.6	1:8 bridge buffer:water
Hb MPI	0.04M citrate adjusted to pH 6.1/6.5 with N-3 Aminopropyl morpholine	1:20 bridge buffer:water
SOD	0.01M citric acid, adjusted to pH 7.0 with tris	1:15 bridge buffer:water

Table 7.5 Electrophoresis running conditions for barn owl blood samples.

Protein	direction of migration	current (mA)	voltage (V)	time (hours)
Tf, Pt, ALB	+	~90-40	190	4
ACP	+	~90-40	190	4
LAP	+	~98-45	190	3
EST-S, Ng	+	~98-45	190	3
PGM	+	50	~125-150	18
GPI	-	50	~125-150	18
MDH	+	~88-80	190	5
LDH	+	50	~30-45	18
6-PGD	+	50	~30-45	18
Hb	-	~50-65	300	3
MPI	+	~50-65	300	3
SOD	+	70	190	5

Table 7.6 Histochemical stain recipes used in protein electrophoresis. (Sigma Chemical Company product numbers in parentheses)

Tf, Pt, ALB	**ACP**
100ml 5:1:5 methanol:acetic acid:water 0.25g Napthol blue black (N3393) incubate for 60 seconds, rinse with water, clear and fix with methanol:acetic acid:water	50ml 0.05M sodium acetate pH 5.0 0.05g Black K salt (F7253) 0.05g Na α napthyl acid phosphate (N7000)
LAP	**EST-S, Ng**
100ml 0.2M Phosphate buffer pH 7.5 0.1g L-Leucyl β napthylamide (L0376) 0.1g Black K salt (F7253)	50ml 0.1M Phosphate buffer pH 7.5 0.1g α napthylacetate (N8505) in a drop of acetone 0.05g Fast blue RR
PGM	**GPI**
40ml 0.05M tris/HCl pH 8.0 0.6g agar 0.6g glucose-1-phosphate (G7000) 0.01g NADP (N3886) 0.5ml $MgCl_2$ @ 20% (M0250) 0.5ml MTT @ 10mg/ml (M2128) 0.5ml PMS @ 10mg/ml (P9625) 30ul Glucose-6-phosphate dehydrogenase (G5760)	40ml 0.05M tris/HCl pH 8.0 0.6g agar 0.04g fructose-6-phosphate (F3627) 0.025g NADP (N3886) 1.5ml MTT @ 10mg/ml (M2128) 1.5ml PMS @10mg/ml (P9625) 20ul Glucose-6-phosphate dehydrogenase (G5760)
MDH	**LDH**
100ml 0.05M tris/HCl pH 9.2 0.4g $MgCl_2$ (M0250) 0.15g NAD (N7004) 3ml MTT @10mg/ml (M2128) 2ml PMS @ 10mg/ml (P9625)	100ml 0.05M tris/HCl pH 9.2 0.2g L-Lactic acid (L1750) 0.12g NAD (N7004) 3ml MTT @10mg/ml (M2128) 2ml PMS @ 10mg/ml (P9625)
SOD	**Hb**
50ml 0.05M tris/HCl pH 8.5 0.02g NAD (N7004) 2ml MTT @ 10mg/ml (M2128) 1ml PMS @ 10mg/ml (P9625) expose to the light for 5 minutes, then incubate in dark until bands appear	110ml methanol:acetic acid:water 5:1:5 0.2g Napthol blue black (N3393) incubate for several minutes, then rinse with methanol:acetic acid:water 5:1:5
MPI	**6-PGD**
20ml 2% agar 20ml tris/HCl pH 8.0 13mg D mannose-6-phosphate (M3655) 5mg NADP (N3886) 30ul G6PDH (G5760) 5ul PGI (P5381) 5mg MTT (M2128) 2mg PMS (P9625)	20ml 2% agar 20ml tris/HCl pH 8.0 50mg $MgCl_2$ (M0250) 250ul MTT @10mg/ml (M2128) 0.0025g NADP (N3886) 0.015g 6-PGA (P6888) 100ul PMS @ 10mg/ml (P9625)

7.2:2 Procedure for starch gel electrophoresis of barn owl blood samples

1ml barn owl blood samples were stored in a -20°freezer as separate red blood cell and plasma fractions; their collection, immediate treatment and storage are described in section 3.4.

Sub-dividing the samples

The first time that a red cell fraction or plasma sample from a particular bird was used in an electrophoresis trial, the sample was thawed at room temperature, and then sub-divided for use in subsequent trials. Batches of 16 samples were thawed at one time, and 30µl aliquots were transferred with a micropipette to the 16 wells of each of five microtitre trays. In this way, five identical trays were prepared for each batch of samples, with the microtitre wells corresponding to the 16 wells of an electrophoresis gel. This procedure reduced the possibility of a loss of enzyme activity through repeated thawing and freezing of samples in a series of electrophoresis trials, and the sample preparation for a subsequent gel simply involved thawing one microtitre tray of samples. The trays were sealed with parafilm and stored at -20°C until required; five minutes of thawing at room temperature was found to be sufficient. One 30µl sample would have been sufficient for six electrophoresis runs, but most were only used for one or two runs; staining the same gel for different enzymes and running gels simultaneously for different enzymes helped to reduce the number of times a set of samples was thawed.

Gel preparation

A mould for each gel was formed by sticking a perspex frame to a perspex base plate using vaseline, and holding the two together with bulldog clips. In a 1 litre side arm flask, 27.5g hydrolysed potato starch (Sigma S-4501), 3g sucrose and 250ml gel buffer solution were heated over a bunsen, swirling constantly, until the solution thickened, and then became thinner and less opaque, usually within five minutes. The gel was then degassed using a vacuum water pump, and poured swiftly and evenly onto the prepared gel plate. This formed a gel 180 * 150mm, and approximately 8mm thick. The gel was sufficiently set to be used within 45 minutes, but could be wrapped in clingfilm after it had stopped steaming, and kept overnight in the fridge. No difference in results was observed according to whether the gel was used immediately or kept overnight, although leaving it for a longer period caused drying and shrinkage, rendering the gel unsuitable for use. The sucrose was included to improve the handling qualities of the gel (Radler, pers. com.).

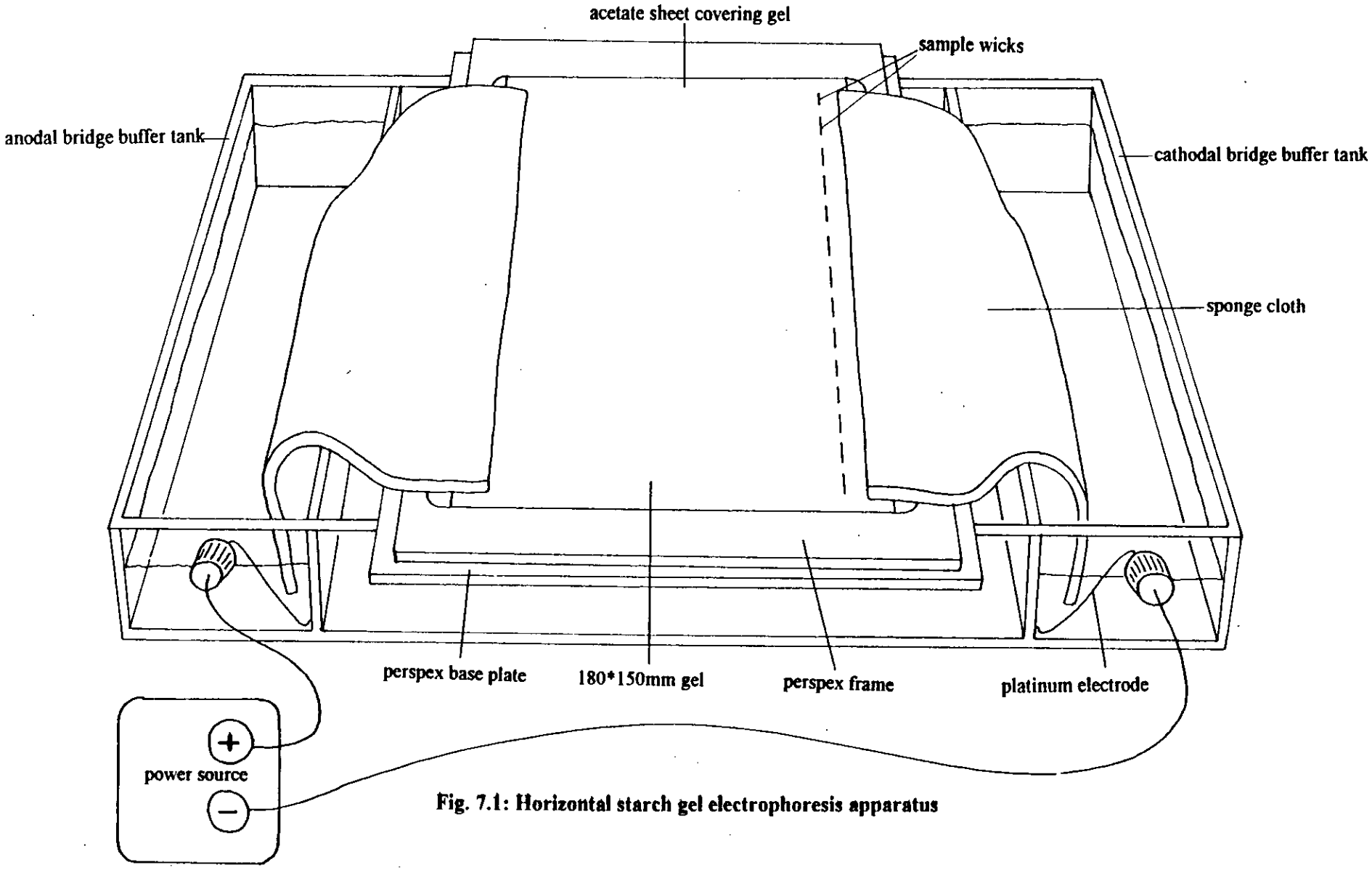


Fig. 7.1: Horizontal starch gel electrophoresis apparatus

Gel running

The electrophoresis apparatus is illustrated in fig. 7.1. Excess gel was cut away from the edges of the set gel, and the bulldog clips were removed; a line of 16 slots was cut with a metal comb, 3cm from one of the long edges of the gel. The 16 thawed samples from a microtitre tray were soaked onto 4 * 4 mm filter paper wicks, which were then inserted into the slots in the gel using fine forceps; wiping the forceps between samples avoided cross contamination. The gel, loaded with samples, was placed between two bridge buffer tanks fitted with platinum electrodes, and each containing 250ml of the appropriate buffer solution. The samples were parallel to the buffer tanks, and usually closest to the cathode connection, by convention on the right hand side. A piece of acetate sheet covered the central area of the gel, but leaving 2cm strips along each long edge to allow the wicks to make contact with the gel; this helped to prevent the gel from drying out, and also encouraged the formation of a straight buffer front. The wicks consisted of household 'sponge cloths', cut to shape to avoid making direct contact with the electrodes whilst covering the whole width of the gel. A layer of clingfilm over the gel and wicks further helped reduce dessication; the apparatus was placed in a fridge to keep it cool during the run. Additional cooling was sometimes necessary, using ice or freezer packs; this depended on the voltage and duration of the run.

Staining

When the gel run was completed and the current switched off, the gel was removed from the fridge and the clingfilm, buffer wicks, sample wicks and gel frame were removed. The gel was cut horizontally in two with a wire gel cutter, and one corner cut to allow later orientation. Each half was then transferred, cut side uppermost, to a glass staining tray or plate.

Staining was either by agar overlay or in solution; in either case, staining ingredients which were purchased in powder form were made in advance into solutions where possible, for ease of handling. Staining in solution occured in 100ml of staining buffer, in glass trays.

Agar overlays were preferred, if staining results were satisfactory, as much smaller quantities of staining ingredients were required. In this case, the gel was placed on a glass plate covered in clingfilm, as the agar prevented any loss of the stain; the gel could be easily disposed of after results were scored.

The gel was covered, and incubated in an oven in the dark (unless otherwise stated in the staining recipie) at 30C for a few minutes to a day, depending on the system.

Gels could be preserved by rinsing and soaking in a solution of methanol: acetic acid: water 5:1:5, and could be sealed in plastic and kept in the fridge indefinitely. Some gels preserved by soaking in glycerol developed discolouring moulds after months of storage sealed in plastic.

7.3: Results of protein electrophoresis trials

Samples

A total of 229 barn owl blood samples were screened by electrophoresis after the systems summarised in tables 7.4-7.6 had been developed; this is lower than the total number of blood samples collected due to the degradation of some of the earlier collected samples, as described above. Table 7.7 summarises the distribution of blood samples among populations, sex and age classes.

Table 7.7 The number of blood samples available for protein electrophoresis from five populations

Population	adult males	adult females	unknown sex adults	juvenile males	juvenile females	unknown sex juveniles	total
1	26	37	0	19	17	17	116
2	14	23	0	4	2	0	43
3	14	11	1	4	0	0	30
4	4	6	10	0	1	7	28
5	4	4	0	1	3	0	12
total	62	81	11	28	23	24	229

Zymogram interpretation

MPI (fig. 7.2) is a monomeric enzyme ie. it consists of two polypeptides. All the barn owls screened (n=229) exhibited identical patterns of two anodal bands. Evans (1987) notes that this enzyme is vulnerable to secondary modification, and the banding pattern is similar to humans homozygous for one MPI locus illustrated in Harris and Hopkinson (1976). This pattern is therefore interpreted as all individuals homozygous for the same allele at a single MPI locus.

GPI (fig. 7.2) is a dimeric enzyme ie. it consists of three polypeptides. The single band shown for each individual is a typical homozygote pattern; as far as can be determined by one dimensional electrophoresis, all individuals were homozygous for the same allele. Haemoglobin is visible cathodally to the GPI bands.

PGM (fig. 7.2) is monomeric, yet the zymograms showed two strong bands with an additional weaker anodal band; all barn owls exhibited the same pattern. This enzyme is vulnerable to secondary modification (Evans, 1987), and so the pattern is interpreted as all individuals homozygous for the same allele at a single PGM locus.

SOD (fig. 7.2) is dimeric or tetrameric (three or five polypeptides) (Evans, 1987). The two strong bands shown for each individual were flanked by two weaker bands, which developed

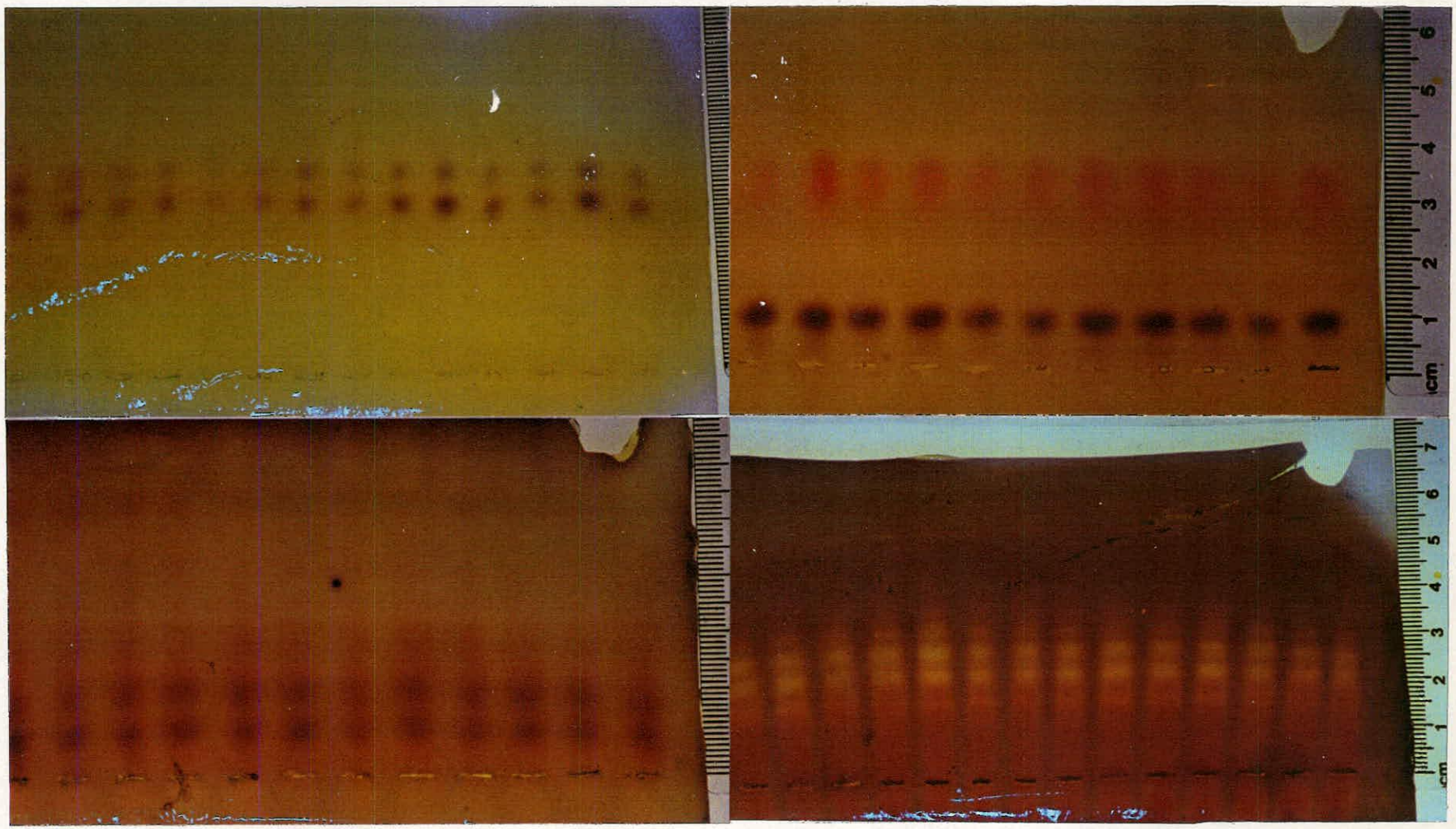


Fig. 7.2: Starch gel electrophoresis zymograms- stained for MPI (top left), GPI (top right), PGM (bottom left) and SOD (bottom right); see text for interpretation of the banding patterns

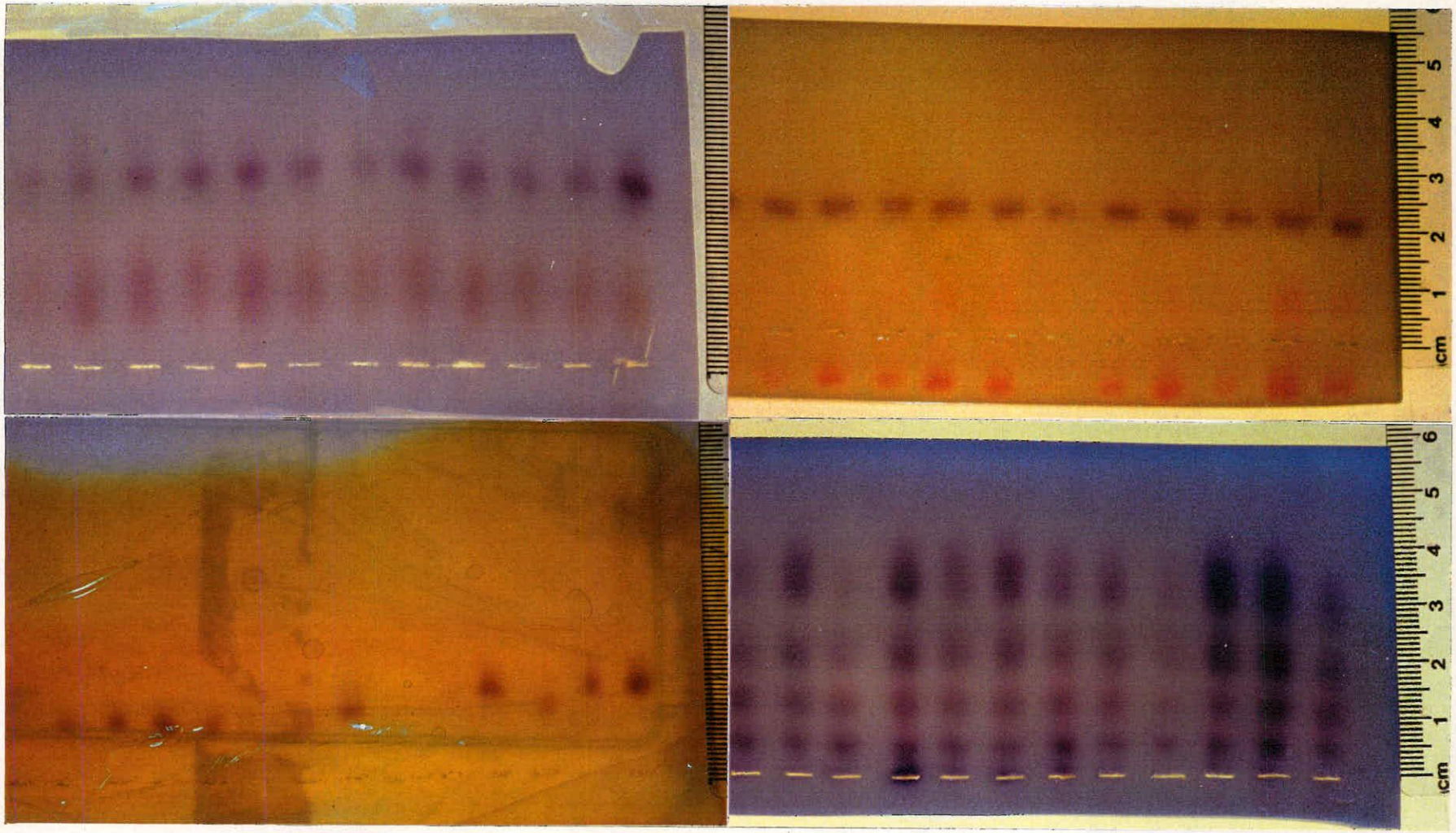


Fig. 7.3: Starch gel electrophoresis zymograms- stained for 6-PGD (top left), MDH (top right), NP (bottom left) and LDH (bottom right); see text for interpretation of the banding patterns

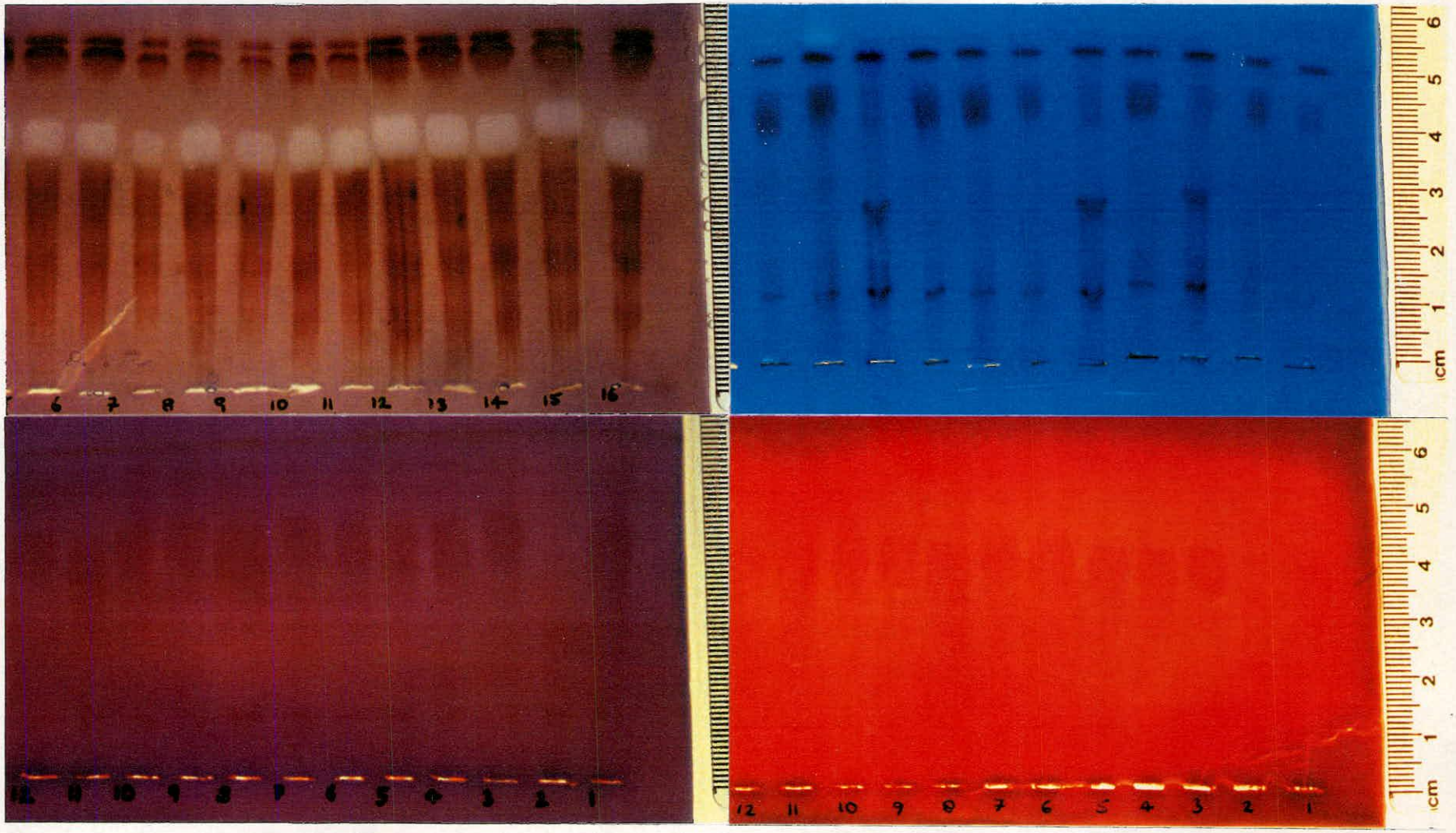


Fig. 7.4: Starch gel electrophoresis zymograms- stained for EST-S, Ng (top left), Tf, Pt, ALB (top right), ACP (bottom left) and LAP (bottom right); see text for interpretation of the banding patterns

more slowly during incubation. No variation in the banding pattern was observed among barn owls; they are interpreted as being homozygous for the same allele at the SOD locus.

6-PGD (fig. 7.3) is a dimeric enzyme; the single row of bands indicates homozygosity, with all barn owls fixed for the same allele. Haemoglobin is visible cathodally to the 6-PGD bands.

MDH (fig. 7.3) is dimeric; as with 6-PGD, all barn owls were homozygous for a single allele, shown by the single row of bands on the zymogram. Haemoglobin migrates cathodally on this system.

LDH (fig. 7.3) is tetrameric; all individuals exhibited five distinct bands in identical arrangement. Harris and Hopkinson (1976) describe five bands as a typical pattern for heterozygosity in tetramers, yet the central bands would be expected to be the strongest, which was not the case on the barn owl zymograms. Evans (1987) describes five bands occuring for homozygous tetramers, with heterozygotes showing eight bands; he notes that LDH may also stain as three or four bands in homozygous individuals. The barn owls are therefore classed as homozygous for the same LDH allele.

EST-S (fig. 7.4) is described by Evans (1987) as monomeric or dimeric; several loci have been described in birds. Two clear bands close to the buffer front headed a further three or four poorly defined bands, which may represent secondary modification of the furthest migrating polypeptides, or could represent additional EST-S loci. Redfield (1973) illustrates EST-S gels on which albumen shows up as a long smear behind the buffer front, which could account for the smearing observed on the barn owl zymograms. No variation was observed among barn owl EST-S banding patterns, but it is not clear how many loci are expressed on these zymograms.

Hb is monomeric, and was monomorphic for these samples, with all individuals expressing a single band. It is not illustrated when stained, where it appears as black bands cathodally on the MPI system, but can be seen fairly clearly on the GPI and MDH gels in figs. 7.2 and 7.3.

The nine proteins described above were monomorphic for all 229 barn owls sampled, representing at least nine different loci, although the exact number of loci involved is hard to determine in non varying systems. Variation was observed in some zymograms, however; these results are discussed below.

NP, nucleoside phosphorylase (E.C. number 2.4.2.1) was the subject of many trials under different electrophoresis conditions, as it appeared in early trials to exhibit different banding patterns among individuals. An example of the highest resolution achieved (with the NP system of Radler, from Harris and Hopkinson, 1976) is presented in fig. 7.3; modifications of this system and a variety of others failed to improve on these results; the resolution was never

clear enough to provide unambiguous results. This enzyme could therefore not be included in the population study.

Ng was first described and named by Birdsall et al., (1970) as white bands which appeared on gels stained for EST-S in natural populations of deer mice *Peromyscus maniculatus* and blue grouse *Dendragapus obscurus*, and they comment that it is also present in other vertebrates. White patches are clearly visible on the EST-S zymogram in fig. 7.4, but unlike the clear bands occuring in the mouse/grouse esterase systems, the appearance of the pattern was not in clearly defined bands, either during incubation or after several hours as illustrated in fig.7.4. The white patches did not all occur at a constant distance from the origin, indicating that this may be a varying system; in blue grouse it was described as a polymorphism at a single locus, with six phenotypes identified, each individual having a maximum of two bands. Exact duplication of Redfield's methods (1973) was not feasible as he employed vertical electrophoresis, but the buffer systems were very similar to those used in this study. Minor adjustments to buffers did not improve the resolution of the white patches on the EST-S gels, however.

Tf, Pt, ALB (fig. 7.4)

In these zymograms, a single band occured close to the buffer front, and another ~12mm from the origin, in all samples (n=229). Between these bands, a single dark staining region occured in the majority of individuals immediately behind the most anodal single band. In a small proportion of individuals, (n=21) this region was more faint, but a stronger single band occured at ~30mm from the origin. Reference to Milne and Robertson (1965) identified the 30mm band as egg white albumen; Tf and Pt are therefore interpreted as homozygous for the same allele in all individuals, although it is not known which protein is represented by which single band. Milne and Robertson (1965) studied egg white proteins in herring gulls *Larus argetatus*, lesser black-backed gulls *Larus fuscus graellsii*, gannet *Sula bassana*, starling *Sturnus vulgaris*, great tit *Parus major* and eider duck *Somateria mollissima mollissima*; none of the individuals tested showed an absence of staining at the albumen position, unlike the observed pattern on the barn owl zymograms. All of the samples tested by Milne and Robertson represented the maternal genotype expressed in the egg white; they found three alleles expressed as single bands in homozygotes and double bands in the heterozygotes. A possible explanation for the absence of staining at the expected position on the barn owl zymograms could be that albumen occurs in detectable quantities in the blood only in females at the time of egg production; the distribution of the albumen band among barn owls was therefore examined in more detail. Table 7.8 lists the details of the 21 birds expressing albumen.

Table 7.8 Details of the 21 barn owls which expressed a ~30mm from origin albumen band on Tf, Pt, ALB gels

Barn owl reference no.	Population	Sex	Ageclass	Year sampled	Month sampled
77	2	female	adult	1991	April
79	2	female	adult	1991	April
82	2	female	adult	1991	April
85	2	female	adult	1991	April
91	2	female	adult	1991	April
93	2	female	adult	1991	April
94	2	female	adult	1991	April
95	1	female	adult	1991	April
97	1	female	adult	1991	April
101	1	female	adult	1991	April
104	1	female	adult	1991	May
107	1	female	adult	1991	May
110	1	female	adult	1991	May
117	1	female	adult	1991	May
211	2	female	adult	1992	April
216	2	female	adult	1992	April
219	2	female	adult	1992	May
220	2	female	adult	1992	May
233	1	female	adult	1992	June
239	1	female	adult	1992	June
241	1	female	adult	1992	June

All the barn owls expressing albumen at the ~30mm position on the zymograms were adult females from the two wild populations, sampled during the breeding season. Testing for a random distribution of the protein between the sexes of wild adults gave a highly significant difference between the sexes: X^2 (with Yate's correction)= 18.03*** df=1, and between wild female adults and juveniles: X^2 (with Yate's correction)= 9.58*** df=1. There was a significant difference between adult females in captive and wild populations, X^2 (with Yate's correction)= 10.41*** df=1, but not between adult females in the two wild populations: X^2 (with Yate's correction)= 2.71, df=1. All the captive birds were sampled from September to December, and were not breeding, whereas all wild adult females were caught in April to

June, when they were breeding. The difference in albumen expression between captive and wild birds may therefore be explained by physiological differences experienced by breeding and non breeding females. Among the wild birds, 52% of breeding females (12 of 23) sampled in April expressed albumen, compared to 24% (6 of 25) in May and 25% (3 of 12) in June; proportionally more females expressed albumen early in the breeding season, when egg production is at its maximum. Although the sample size is small, these results are consistent with the hypothesis that albumen presence in the blood is of a transient nature, expressed in detectable quantities in females which are producing eggs. This is also supported by the pedigree data, as of the 21 birds listed in table 7.4, offspring were screened for six of them (see appendix 1 for pedigree data), making a total of 2 female and 10 male offspring, none of which expressed the albumen band. It should be noted that not all breeding females expressed ALB, however; 39 of 60 did not express the band. This may be due to the protein only occuring in detectable quantities in the blood at very specific stages of egg production, but this remains uninvestigated in this study.

ACP and LAP

These zymograms were identical to the patterns produced for Tf, Pt, ALB (fig. 7.4) for every individual sampled (n=229), although staining was more faint in the majority of cases. An association between enzymes could explain these results, if electrophoresis separated enzyme complexes rather than individual polypeptides, such that enzymes were held together as they migrated through the gel to be detected by their specific stains at the same positions. This is inconsistent with the observed banding pattern, however, particularly as one band appears to be albumen present only in breeding females. It is therefore assumed that these zymograms are repeats of the Tf, Pt, ALB system, and that staining for ACP and LAP was not specific.

Heterozygosity and polymorphism in this study

Eleven of the proteins screened by starch gel electrophoresis were monomorphic (MPI, GPI, PGM, SOD, 6-PGD, MDH, LDH, EST-S, Hb, Tf, Pt). The number of corresponding monomorphic loci may be higher, as more than one locus may be represented on the EST-S gels.

Of the other proteins screened, 'ACP' and 'LAP' are discounted, as they appear to have stained for the Tf, Pt, ALB system; one albumen band was expressed in only 21 individuals, but appeared monomorphic, and two possible varying systems were never adequately resolved to quantify any variance.

It is therefore concluded that barn owls in this study expressed levels of heterozygosity and polymorphism of 0, calculated over 11 loci, but that the inclusion of further proteins could alter this figure.

7.4: Discussion

The expression of albumen exclusively in breeding female barn owls serves to illustrate some of the hazards inherent in isozyme interpretation. Evans (1987) stressed the importance of testing the genetic basis of allozyme patterns by comparing parents and offspring; in his study of starlings *Sturnus vulgaris*, parents were caught as adults, but offspring were sampled in the nest. For a protein such as albumen, which appears to be only transiently present in the blood of adult females, expected inheritance patterns in parents and offspring would have led to the conclusion that the bands had no genetic basis, whereas the discrepancy may have been a function of age and physiological condition. Such inheritance testing should therefore be restricted to birds sampled in the same physiological state; this may be particularly relevant to studies of blood proteins, as the blood transports the products of genes which may be active elsewhere in the body. If the albumen data had been examined without reference to the details of the birds which expressed the bands, it could have been concluded that it represented a genetic difference between captive and wild birds, which could in turn have led to speculation about higher levels of inbreeding in the captive birds, as none expressed the 30mm band, whereas it was expressed in 21 of the wild birds. The population differences may be explained, however, as an artifact of the time the birds were sampled, as none of the captive birds were sampled when breeding, whereas all the adult wild birds were sampled during their breeding season. Isozyme studies which amalgamate birds of different ages, sexes, and make comparisons of levels of variation when different loci have been screened in each population may therefore be subject to a number of biases and confounding effects, and such inter-population comparisons should be confined to cases where such factors are taken into account and minimised.

As a relatively small number of loci were screened in this study, it may seem inappropriate to compare these results with those from other avian studies which have included far larger numbers of loci. Although it could be misleading to compare single estimates of polymorphism or heterozygosity among studies including large differences in the number and identity of loci screened, a useful comparison with other studies can be attained by a locus by locus approach. Evans (1987) summarises the frequency of polymorphisms in specific enzymes in avian studies, including 11 proteins included in this study. 50% or more of the species reviewed (from 29-83 species per protein) were polymorphic for EST, PGM, MPI, Tf and 6-PGD; 25-50% of species were polymorphic for GPI (n=36) and NP (n=23), and up to 25% were polymorphic for ALB, Pt, ACP and LDH (n=26-77). Hence the barn owls were monomorphic at 5 loci which were polymorphic in more than half of the species studied; this comparison supports the idea suggested by the overall lack of polymorphism in the proteins studied, that they have lower levels of variation than would be predicted on the basis of other

avian studies. Comparing these results with those of Randi et al., (1991), barn owls in their study were also monomorphic for MPI, LDH, GPI, PGM, EST, MDH, Pt and ALB; two enzymes, AAT and ME were polymorphic by electrophoresis on polyacrylamide gels, but these were not assayed in this study, and so possible differences between the British populations and those from Randi et al.'s study in Italy, presumably also of *T. a. alba* remain unknown.

Low levels of variation may partly be accounted for by taxonomic differences, but may also be due to past demographic patterns. Thompson et al. (1991) studied serum esterases by chromatofocussing, and found fewer forms of the enzyme in the barn owl, little owl, tawny owl and razorbill than in starlings, house sparrows, tree sparrows, pigeon, partridge and magpie. They correlate this difference with the diet of the species, the carnivores having fewer EST forms than the omnivores and herbivores; a relationship which could be causal, given that EST is involved in digestion. Barn owls may therefore exhibit lower isozyme variation than other birds for ecological reasons, in which case it would be expected to be consistent among populations and even sub-species. Particularly low levels of variation in native wild barn owls could also be indicative of a past population bottleneck; as described in chapter 2, Britain was colonised by barn owls soon after the end of the last ice age, around 10 000 years ago. If initial population sizes were low, isozyme variation would be reduced through drift, and present day isozyme patterns may reflect the events of this distant period, as it would take many more generations to recover to former levels of variation. A comparison of continental and British barn owls would be informative on this issue.

Randi et al. comment that protein electrophoresis may be used to investigate phylogenetic relationships at higher levels in birds than in other taxa, due to the short genetic distances among taxa. Praeger et al. (1974) demonstrated that Tf and ALB from birds had evolved more slowly than in other vertebrates, and suggest a general evolutionary slowdown at the molecular level in birds; possible explanations involve the characteristic high and stable body temperature of birds, or a low DNA content per cell. In this study of barn owls, a lack of population differentiation suggests that protein electrophoresis may be more appropriately used comparing more divergent groups, such as among sub-species or populations separated by a greater geographical distance. It would be particularly interesting to compare British and continental *T. a. alba*, through the hybrid zone with *T. a. guttata* in France; this remains a possibility for future study.

The implications for conservation management of the lack of protein variation found in this study are discussed in chapter 8.

SUMMARY

Genetic variation in blood proteins was investigated by the technique of starch gel electrophoresis. Systems were developed by empirical modification of previously published protocols to give banding patterns for 15 blood proteins in barn owls; 229 barn owl blood samples from the two wild and three captive populations were then tested for isozyme variation.

Of the 15 proteins assayed, 11 (MPI, GPI, PGM, SOD, 6-PGD, MDH, LDH, EST-S, Hb, Tf, Pt) were monomorphic in all 229 samples. ACP and LAP were rejected as their banding patterns were identical to the distinctive pattern of Tf, Pt, ALB gels; Ng was not included due to poor resolution of the bands, and ALB was expressed in only 21 individuals.

A significant difference in the expression of ALB between captive and wild barn owls could be explained by the time of year at which sampling took place; it was expressed exclusively in breeding females, and no captive females were breeding when sampled. The need for caution in interpreting isozyme data is therefore stressed, as birds sampled in different physiological states may have different proteins circulating in their blood.

The barn owls in this survey showed no polymorphism at the loci studied; although a relatively small number of loci were tested, they included five proteins which have been found in other avian studies to be polymorphic in over 50% of the species reviewed. Low levels of protein variation could be explained by taxonomic characteristics of the Strigiformes, or by past demographic events such as a bottleneck.

Chapter 8

REVIEW AND CONCLUSIONS

8.1: The case for genetic monitoring

The possible genetic consequences of a declining population of barn owls were outlined in chapter 1 as an increase in inbreeding and a loss of genetic variation, resulting in a reduction in the population's potential to adapt to a changing environment. In chapter 2, the available demographic data were reviewed; it was concluded that initial genetic concerns were justified, as barn owls are experiencing a widespread decline throughout the country, with habitat deterioration and fragmentation resulting in sub-populations becoming increasingly isolated.

One approach to evaluating the risks of these processes to the populations' short- or long-term survival involves predictions based on genetic parameters such as mutation rates and the effects of drift, and population parameters such as the population size, mating system, fecundity and mortality. Simulation models for population viability analysis (PVA) such as the VORTEX program, for instance, allow population persistence to be modelled under different scenarios of random and deterministic processes (Lacy, 1993). The program produces an estimated probability of extinction as time progresses, based on parameters supplied by the user; habitat decline can be incorporated in terms of falling carrying capacity, and random 'disasters' can be included. Genetic options allow the user to specify the severity of inbreeding depression for a given loss of heterozygosity (Caughley, 1994).

Unfortunately, this approach is only valuable where reliable demographic data for the species of interest are available, yet such data are commonly not available for those rare or endangered populations for which accurate predictions would be of most use (Caughley, 1994). This criticism could certainly be relevant in the case of wild barn owl populations, for which it is notoriously difficult to obtain accurate census data (chapter 2). Barn owls are secretive and nocturnal, distributed at low density, and populations naturally fluctuate in accordance with three to four year fluctuations in prey availability. Although an intensive long term local population study such as Taylor's (1994) may provide accurate figures for fecundity for that population, it is not necessarily appropriate to extrapolate these figures to populations in different areas of the country, which may experience different environmental conditions such as habitat types. In addition, natal to breeding site dispersal distances may be underestimated by a local study, but overestimated from ringing

return data, as described in chapter 2; mortality rates in the local study may incorporate an error if mortality can not be distinguished from dispersal out of the study area. Population theory predicts that very low levels of interpopulation mixing (in the order of one migrant per generation) can counter the effects of drift in sub-populations. Without extremely accurate dispersal data, therefore, the effective population size cannot be estimated with any confidence.

Such sources of error in population viability analysis are likely to be common in rare or endangered populations, and so in addition to obtaining census data, the direct monitoring of genetic variability is recommended where possible in long term population management plans. Lande and Barrowclough (1987) suggest protein electrophoresis and quantitative traits as suitable techniques for population monitoring.

8.2 The challenges of acquiring genetic data from rare or endangered species

Protein electrophoresis has been successfully employed to examine genetic variation in a number of avian studies (see chapter 7), and quantitative traits are widely studied in birds; the heritability of quantitative traits has been estimated in several studies of wild bird populations (see chapter 6). There is a bias in the published literature, however, in favour of those species for which the required data are easily obtained. Protein electrophoresis requires a tissue sample, and the data may still be informative even when a small number of individuals are sampled; 20-30 individuals are considered adequate for estimates of average heterozygosity, for instance (Lande and Barrowclough, 1987). If the heritability of quantitative traits is to be estimated, however, larger samples of related individuals are required, preferably sampling families consisting of both parents and their offspring. In some avian studies, the birds are culled to provide tissue samples and morphometric data (see Evans, 1987), or museum specimens are utilised, as in Earhart and Johnson's (1970) study of sexual dimorphism in various owl species. Heritability studies favour those populations for which large samples of related individuals can be conveniently obtained, as in studies of nest-box dwelling populations of small passerines (see chapter 6). Obtaining these kinds of data from a rare or endangered population is likely to be far from straightforward, however, and the value of these approaches for conservation management depends in part on how feasible it is to obtain the data from populations which present particular challenges for data collection. Sampling such a population must give high priority to the individuals' welfare, for instance; disturbance should be minimised and destructive sampling is inappropriate. In addition, the population may be small, dispersed, and individuals may not easily be located, captured and sampled.

The wild barn owl populations in this project exemplify some of the problems that may be anticipated in other genetic studies where the target species is selected because of the potential value for conservation management of the genetic data, rather than because of its likelihood of providing good results. Barn owls are a protected species in Britain, and their predicament has been given a high profile by various conservation organisations. Farmers and landowners are therefore often understandably protective of the birds that nest on their land, and any research project must therefore obtain not only the appropriate licences to allow roost and nest sites to be visited, but the co-operation of locals must also be ensured. Where nest sites are located in close proximity to occupied dwellings, as is often the case with barn owls, site visits may have to be individually arranged in advance. Nest sites are often not easy to locate, being secreted in tree holes, under the eaves of old buildings, or down disused chimneys; once located, they may prove inaccessible or dangerous to approach due to the state of repair of the building. Capture of adults may be effected by use of a hand held net, as described in chapter 3, but the roost or nest site must be approached in silence if the birds are not to be flushed before the nets are in place; this operation may require ladders to reach upper windows and several assistants may be required at some sites to cover all possible exits from a building. As not all the sites are easily accessible by road, and may be several miles apart, site visits may be extremely time consuming. Although adult females may be reliably caught from the nest by this method, males roost at the nest site for a far shorter period of the breeding season, and so netting may prove ineffective for sampling the males. Barn owls hatch asynchronously, and so several site visits per brood would be required if they were to be sampled at constant age.

8.3 Success of data collection in this study

These characteristics of barn owl populations demonstrate that sampling the birds is not a simple or straightforward process, yet in this study many of the problems were overcome by a concentration of time and resources on one of the wild populations. In the Langholm population (population 1), a large number of potential nest sites were known because of Taylor's (1994) long term population study, and land owners and farmers were generally already amenable to the study of the birds on their property. Around 70 potential sites were visited before the onset of the breeding season each year, and so occupied sites were known early in the season. Where feasible, sites were provided with large, specially designed nest boxes positioned before the onset of breeding (see chapter 3), and I designed and constructed a box-trap which enabled adults to be captured when they entered the nest box to feed their young. Although time consuming (one night per bird caught) this method enabled adults to be caught at some sites which would not otherwise have been possible. Young barn owls were sampled in the nest once their skeletal growth was complete, but

not at constant age, to minimise the number of site visits required. Sampling of each bird involved taking a 1ml blood sample, photographs of plumage and morphometric measurements. A great deal of data were therefore obtained from a single sampling event per bird, for less than 15 minutes handling time per individual. The frequency of site visits, and handling the barn owls, was in accordance with the methods employed by Taylor in his long term study of this population; he had determined that his procedures were not detrimental to the birds (see chapter 3).

Despite the considerable difficulties in obtaining genetic data from the wild barn owls, the intensive approach to data collection described above proved to be effective in this study. Blood samples were obtained from 116 individuals from population 1, of a total of 229 from the five populations combined. Morphometric data was collected from all individuals. This included family data from 114 individuals in population 1, distributed among 30 families with up to seven offspring per family, and one or both parents (detailed in appendix 1). Several factors contribute to the difficulty of obtaining good estimates of heritability in wild populations; as outlined in chapter 6, large errors are often associated with estimates derived from small sample sizes, and bias is introduced by often unquantifiable and uncontrollable environment and genotype correlations. Despite these problems, the heritability estimate of 0.64 ± 0.27 for tarsus length by mid-parent - offspring regression demonstrates that this approach to estimating heritability may be as effective in a species difficult to study, such as the barn owl, as it is for species more amenable to study (see chapter 6), if a considerable effort is applied to the collection of data.

8.4 Evaluating the techniques

Having demonstrated the feasibility of sampling barn owl populations to obtain samples for protein electrophoresis, and to obtain morphometric data suitable for heritability analysis, the merits of these two techniques in this study can now be compared.

The incentive to pursue an isozyme study stems from the nature of the genetic information attainable, if variation is found at the intra or inter-population level. Isozyme banding patterns can be directly interpreted as genotypes, and so data on inter-population differences in allele frequencies can be rapidly and relatively cheaply generated from a single small tissue sample per individual. This lack of ambiguity in the genetic interpretation of the data allows very precise monitoring of a population for changes in allele frequencies over several generations, or for detecting inter-population differences.

Sampling error is minimised through sampling a large proportion of the population, but isozyme data can still be informative even when a small number of individuals are sampled; 20-30 individuals are considered adequate for estimates of average heterozygosity, for instance (Lande and Barrowclough, 1987).

The drawback of using protein electrophoresis to investigate genetic variation at the population level is that the technique is of little use for interpopulation comparisons or for monitoring change over time if none of the systems exhibit any polymorphism, yet it is not possible to predict with any accuracy whether appreciable levels of isozyme variation will be present without a detailed knowledge of long term demographic patterns in the populations of interest. Despite theoretical reasons for predicting low levels of isozyme variation in raptors, for instance, data from Peregrine falcons *Falco peregrinus* (Morizot, 1988), and eagle owls *Bubo bubo* (Radler, 1992), demonstrate that this is not universally the case.

A pilot study to assess the suitability of the technique is therefore always required; the problem here is that the empirical testing required to develop isozyme systems for a previously unstudied species may be time consuming. Starting with systems developed for taxonomically close species is not necessarily a short cut to achieving good results; this is illustrated in this study, where systems for superoxide dismutase developed for eagle owls and other avian species (Evans, 1987) were rejected in favour of a bryophyte system (Hofman, 1991). In this study, electrophoretic systems were developed to give clear, repeatable results for 12 proteins, 11 of which (MPI, GPI, PGM, SOD, 6-PGD, MDH, LDH, EST-S, Hb, Tf, Pt) proved to be monomorphic in the 229 blood samples. The only protein variation found was an additional albumen band exclusive to 21 wild adult females, and this was discounted from estimates of polymorphism as an artefact of physiological state. Polymorphism and heterozygosity are therefore estimated as zero in the proteins screened, and so this method did not prove useful for monitoring population change or differentiation in this study.

The attraction of a study of quantitative traits is that many quantitative traits are of known ecological importance, and so variation in these traits is directly relevant to conservation; data are readily obtained from live birds sampled in the field. In this study, the data which were collected during the short handling period of each bird were analysed to address a number of different questions. Photographs of plumage enabled sexual dimorphism in plumage patterns and colouration to be examined in detail, and were utilised to assign a sex to juvenile birds for which breeding data could not be obtained. Sexual dimorphism in

tarsus length, wing length and weight was investigated, and in contrast to the published literature for another sub-species of barn owl, the birds sampled showed significant dimorphism in weight only. Patterns of fluctuating asymmetry in the bilateral traits tarsus and wing length were investigated, and a possible index of body condition, using tarsus length to correct for skeletal size in body weight, was compared to a quite different method of assessing condition based on a blood parameter.

Unlike the easily interpreted isozyme data, however, the main problem in a study of quantitative traits involves interpreting the observed patterns of variation; unless the sources of the variation are taken into account, the meaning of observed patterns of variation remains obscure. Fluctuating asymmetry, for instance, may be inflated due to an increase in the environmental component of variance or due to a reduced genetic capability of compensating for environmental perturbations; sexual dimorphism may vary according to the trait studied because of different sources of variation among traits.

One approach to aid the meaningful interpretation of quantitative data is to control for some sources of variation by analysis within categories of a strictly sub-divided data set. Analysis may be confined within sex, age, population or season of sampling categories, for instance, as was recommended for the condition estimates described in chapter 5. The problem with this approach is that the initial data set must be large if reasonable sample sizes are to be maintained within each sub-set.

The approach described in chapter 6 is to attempt to partition observed phenotypic variability into additive genetic and environmental components by heritability analysis. Despite the suitability of tarsus length for heritability estimation in this study, as determined in chapter 5, the heritability estimates are still associated with a high standard error. This is a common feature of heritability estimates in natural populations, as sample sizes are typically smaller than those used in heritability studies of controlled populations (see chapter 6).

The error associated with heritability estimates has serious implications for the use of quantitative traits in monitoring genetic change or detecting genetic differences among populations; the technique is potentially far less sensitive than an isozyme study in detecting significant genetic differences among populations.

Isozymes were initially selected for trial in this study because of their relative cheapness compared to other techniques of assaying genetic variation, and because suitable levels of

variation have been described for assessing variation at the population level in a number of avian studies, including eagle owls (Radler, 1992).

Because large errors are commonly associated with heritability estimates of quantitative traits in natural populations, as demonstrated in this study, they are of limited use for monitoring subtle changes in genetic variability over generations. A method of assaying genetic variation directly using markers more variable than isozymes is therefore desirable for population monitoring; microsatellites may prove suitable in this case, but this was beyond the scope of the project.

8.5 Interpreting the levels of variation

What, then, can be learnt from the levels of genetic variation in barn owl populations as revealed by protein electrophoresis and the quantitative traits in this study?

Comparing the isozymes in this study locus by locus with Evans' (1987) review on polymorphism and heterozygosity in avian studies suggests a general low level of isozyme variation in this population of barn owls in comparison to other birds, but although there are exceptions, several studies suggest that low levels of isozyme variation may be common in raptors (see chapter 7).

Low levels of isozyme variation may be attributable to a period of inbreeding after a population bottleneck, possibly in the distant past (Lande and Barrowclough, 1987); the lack of variation in the barn owls studied could even be a result of inbreeding as far back as the period when Britain was first colonised by barn owls at the end of the last glaciation, some 10 000 years ago. Alternatively, the present lack of detected variation may reflect a naturally low equilibrium level of isozyme variation (Negro and Hiraldo, 1994). A low level of isozyme variation in raptors would be consistent with the pattern described for large, terrestrial predators (Merola, 1994), and may be expected in species with a naturally low N_e. The lack of isozyme variation is therefore not necessarily indicative of an overall depletion in genetic diversity, and is not in itself a cause for concern in population management.

As for variation in quantitative traits, the genetic variability of barn owl tarsus length, as described by CV_A, lies in the middle of the range of the published values reviewed in chapter 6; it does not appear significantly lower in *T. alba* than for populations in which genetic adaptation for quantitative characters has been demonstrated in the wild (Boag, 1983). There is therefore no indication in this self-sustaining population of *T. alba* that

levels of quantitative variation are too low to permit future genetic adaptation for tarsus length; despite an apparent lack of isozyme variation, the population may still maintain sufficient genetic variation in quantitative traits to adapt in a changing environment.

8.6 Conclusions

In summary, there was no evidence from the protein electrophoresis data or morphometric data in this study that the wild barn owl population studied in detail in this project exhibited levels of genetic variation that would prompt special recommendations for genetic management. A review of the available demographic data suggested that isolation may not be as complete as was initially thought for population 1 in this study, and if this is the case throughout the country, genetic considerations are unlikely to require any special priority in population management. Management policies aimed at redressing the causes of the decline in terms of habitat improvements and nest site provisioning are likely to automatically cover any of the perceived genetic problems, unless small fragments of the whole population become completely isolated. Genetic issues are of most relevance in barn owl conservation in the management of captive breeding programmes; strict management to minimise inbreeding in the captive populations, and excluding other sub-species of *Tyto alba* from any breeding programmes are to be strongly recommended, and the legislation governing barn owl releases now enables co-ordination and control of such practices.

In conclusion, this study has demonstrated the feasibility of obtaining quantitative data suitable for heritability analysis in a species which in many ways is not ideally suited for this kind of investigation, but the difficulties in obtaining data in the field in this study suggest that this method of assessing genetic variation has limited practical use for conservation management of rare or endangered species. Although obtaining blood samples for genetic analysis by protein electrophoresis or by some other method may give useful data from a relatively small number of individuals, and avoid many of the sources of error included in the heritability estimates of quantitative traits, these approaches remain invasive, time consuming and costly. Where resources are limited, attempts at directly monitoring genetic variation should not be made a priority in conservation management; management should in preference make informed decisions based on conservation genetic theory. Direct monitoring of genetic variation in example wild populations would be valuable, where possible, to confirm or challenge the broad generalisations of the theory, but is not to be either expected or recommended for every endangered species for which population management is contemplated. It should be remembered that genetic issues are often not the most pressing in conservation; Caughley (1994) warns that 'genetic thinking

often intrudes where it is not relevant and where it sometimes obscures the real issues'. In a small wild population, demographic and environmental stochasticity are far more likely to be the cause of extinction than inbreeding or drift, and so populations managed successfully with the goal of preventing extinction from these causes are likely to automatically avoid any additional genetic problems.

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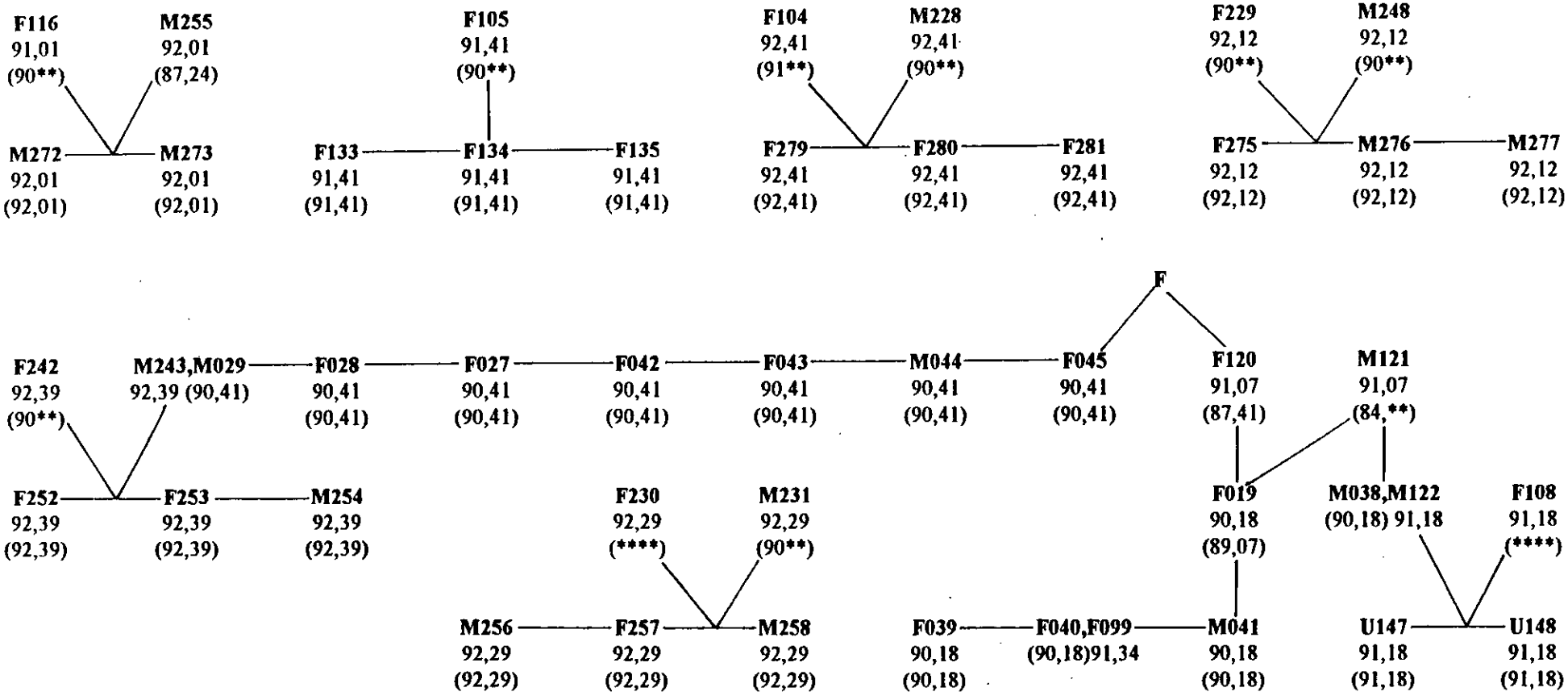
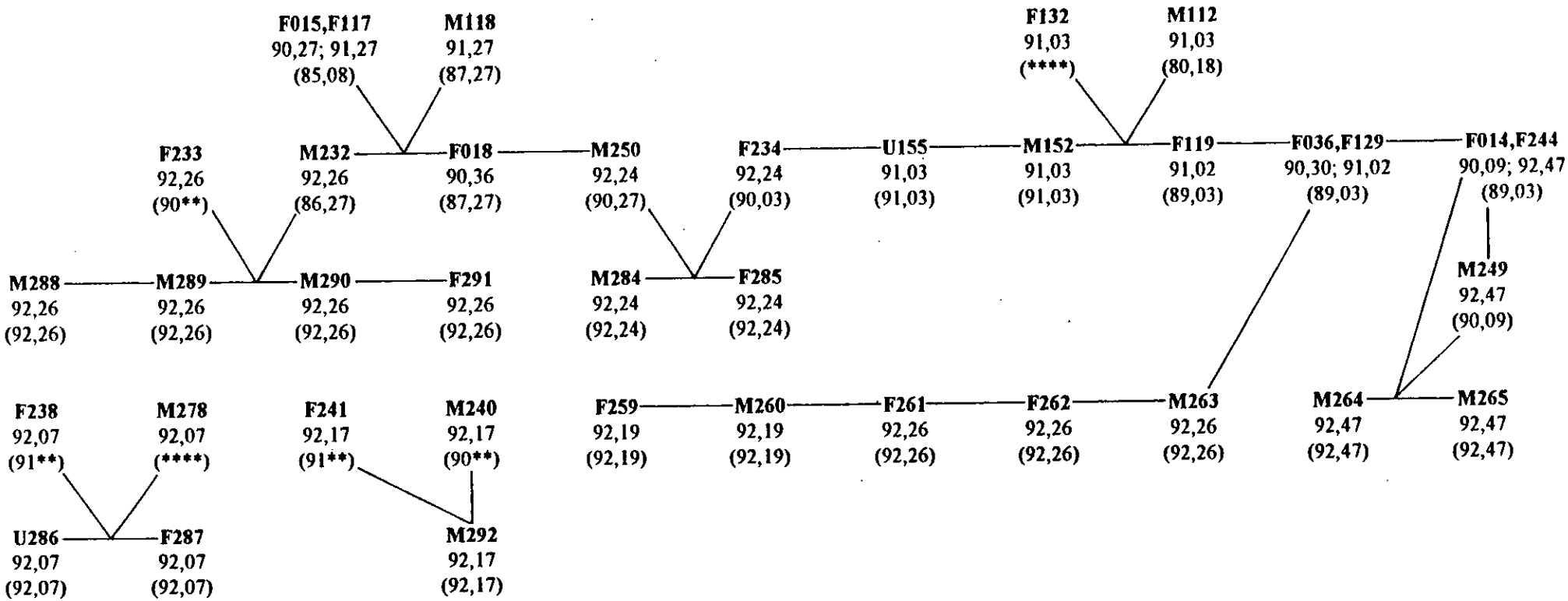
APPENDIX 1 :Pedigrees of related birds within population 1

M= male, F= female, U= unknown sex, 123= sample reference number
91,23= sampled in 1991 at site number 23
(90,21)= fledged in 1990 at site number 21; (****)= data not available



APPENDIX 1 (cont.):Pedigrees of related birds within population 1 (cont.)



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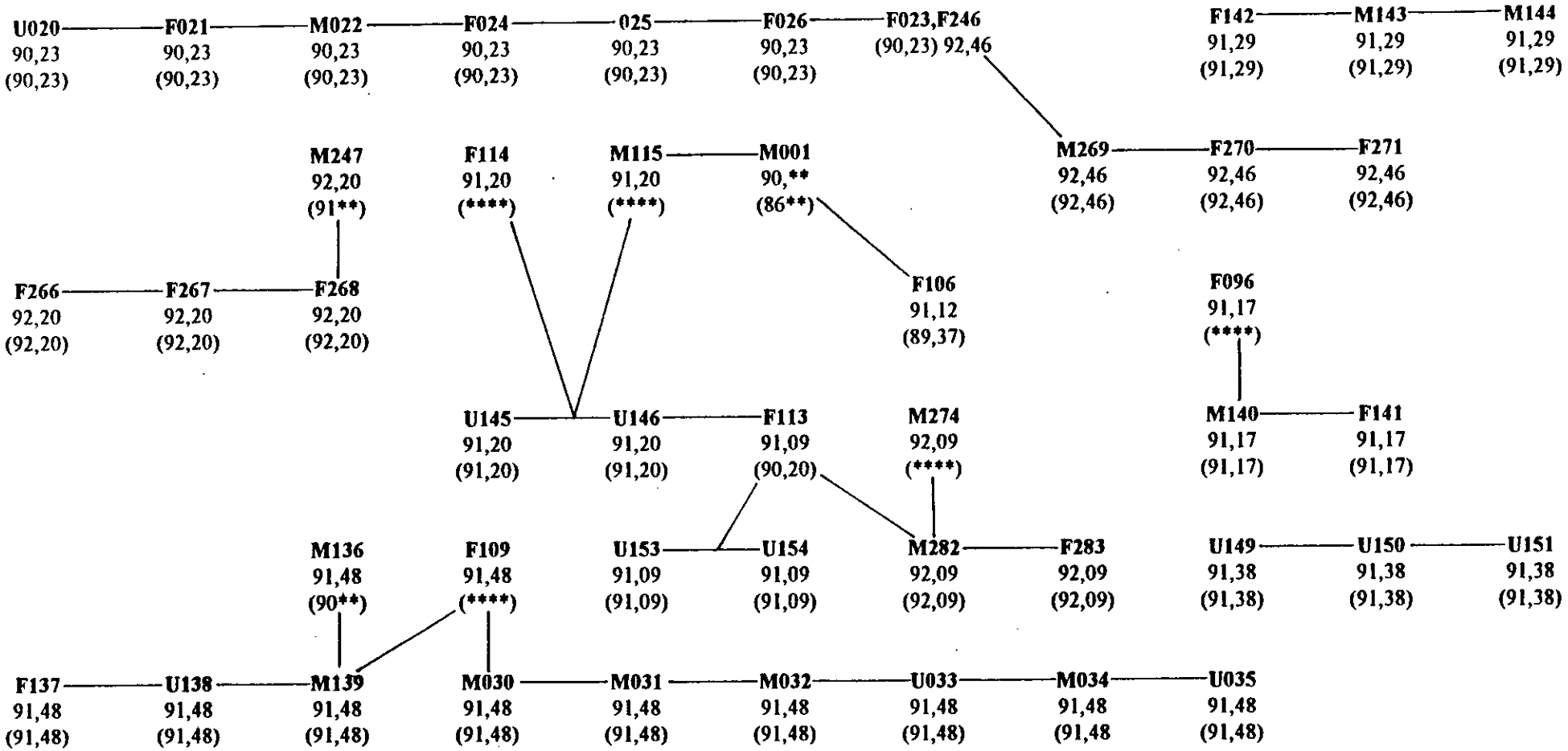
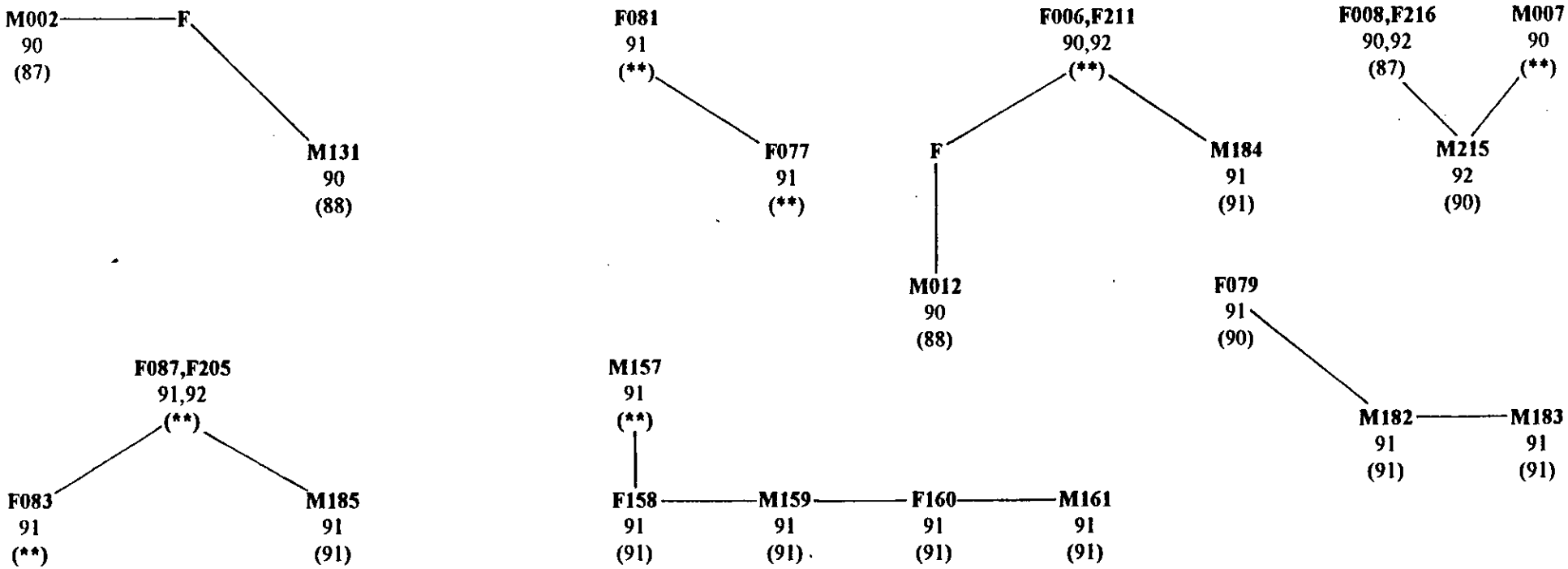
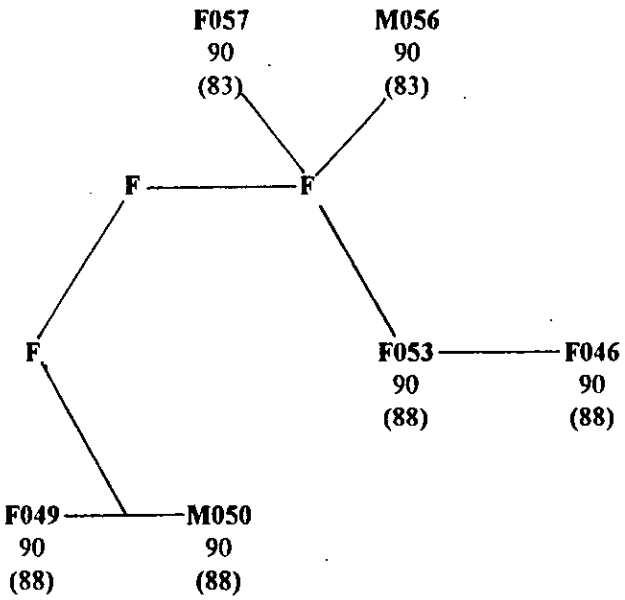


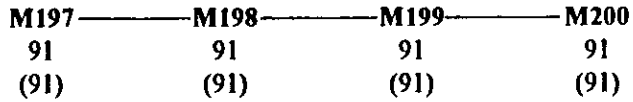
APPENDIX 1(cont.) :Pedigrees of related birds within population 2

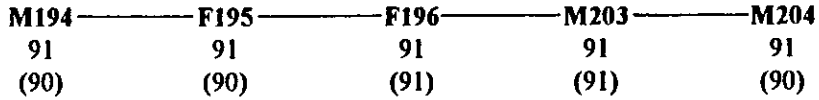
M= male, F= female, U= unknown sex, 123= sample reference number
91= sampled in 1991
(90)= fledged in 1990; (**)= data not available



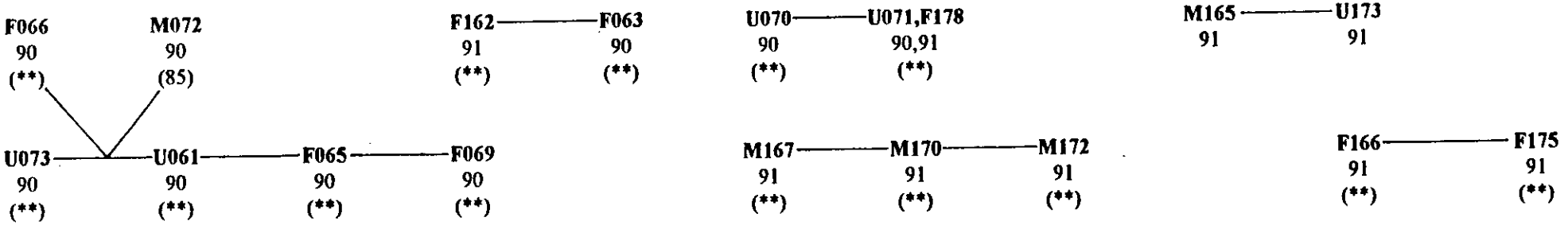
APPENDIX 1 (cont.):Pedigrees of related birds within population 3



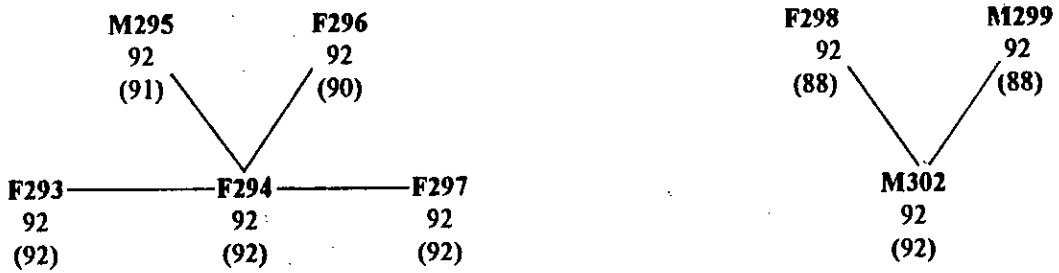




APPENDIX 1 (cont.):Pedigrees of related birds within population 4



Pedigrees of related birds within population 5



Appendix 2

A potential method for determining the sex of barn owls from plasma samples

Chromatographic separation of steroid hormones: methodology

15 blood samples from chickens, turkeys and barn owls were available for trial; these had been sampled and stored as described in chapter 3.

Based on Dieter (1973): The plasma sample (typically 0.5ml) was extracted three times with an equal volume of absolute chloroform plus one-twentieth volume of 1M sodium hydroxide. The extracts from each individual were pooled in a 10ml test tube and evaporated in a waterbath at 40^0C to 100µl.

A chromatographic column was prepared as follows: 3g of a fine particle silica was mixed thoroughly with 80ml ethyl acetate: cyclohexane (1:1 by volume). 4ml of this mixture was transferred with a syringe and canula to the filling head of the chromatographic apparatus made to the specifications of Ribi et al. (1970), and centrifuged for 7 minutes at 1500G. The surplus solution was discarded from the tube, and the top few mm of the packed column removed.

The plasma extract, soaked onto a cotton wool plug, was firmly inserted at the top of the column, and 3ml of developing solvent (1:1 ethyl acetate: cyclohexane) was added to the filling head of the apparatus. It was then sealed and centrifuged for 5 minutes at 2500G.

The silica column was extruded with a rod onto a metal tray, and dried for 10 minutes at 65^0C. The dried column was then transferred to a sealed 10ml tube containing iodine crystals, and incubated for up to several days.

Results of chromatographic trials

Dieter (1973) described five distinct bands being diagnostic of males, females having only two or three. No bands developed in any of the trials. The time of exposure to iodine was not stated in the paper (Dieter, 1973), but chromatograms left for several days in my trials failed to reveal any bands.

Comments on the chromatographic separation of hormones as described by Dieter (1973)

Dieter (1973) does not state in his paragraph on the extraction and concentration of steroid hormones, what volume of blood was initially taken, merely that the plasma sample is

reduced to 100µl. I therefore used a volume of approximately 0.5ml plasma, as this was the amount surplus to my requirements for isozyme analysis from an initial volume of 1ml blood. Although Dieter states that his methods require 'only a small plasma sample', he mentions the use of 4ml plasma from Japanese quail, for the preparation of sulphuric acid chromogen spectra (p3, Dieter, 1973). This would correspond to a blood sample of 6ml or more, depending on the efficiency of separation- by no means a small sample from such a bird, suggesting that samples taken on different occasions may need to be pooled to make up the required volume for an individual. The iodine stain is claimed to be suitable for detecting the presence of 50 nanograms of testosterone and β-estradiol, and this amount was presumably present in Dieter's males, as one of the iodine induced bands was attributed to testosterone. Given that expected testosterone concentrations in birds lie in the region of up to 783 nanograms/100ml (Domestic fowl: Lake and Furr, 1971, cited in Dieter, 1973), it may be speculated that the starting volume of blood should be more than 6ml if testosterone is to be detected.

Dieter (1973) provided no information on sample sizes, species or age of individuals assayed, nor were photographs of the resultant bands published, which did not facilitate the assessment of his technique. As in any case a volume of blood greater than I had available from the barn owls was probably required, no further trials were attempted using this method.